Interaction entre démographie et génétique dans les petites populations : études sur un Hyménoptère parasitoïde avec incompatibilités génétiques

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Interaction entre démographie et génétique dans les petites populations : études sur un Hyménoptère parasitoïde avec incompatibilités génétiques

par

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RÉSUMÉ

Des processus génétiques et démographiques peuvent entraîner l'extinction des petites populations. Les deux types de processus sont souvent étudiés séparément. Pourtant, leur interaction peut générer des vortex d'extinction, comme celui susceptible d'affecter certaines espèces d'Hyménoptères. Les Hyménoptères sont haplodiploïdes : les mâles sont haploïdes et les femelles diploïdes. Chez les espèces avec sl-CSD (single-locus complementary sex determination), les haploïdes, hémizygotes au gène du CSD, se développent en mâles et les diploïdes hétérozygotes, en femelles. Les diploïdes homozygotes au gène du CSD sont des mâles non viables ou stériles. Des études théoriques suggèrent que la production de mâles diploïdes associée à la stochasticité démographique et environnementale peut entraîner les petites populations d'Hyménoptères dans un vortex d'extinction.

Le premier objectif de cette thèse est d'encourager le dialogue entre génétique et démographie en proposant un concept qui regroupe les deux types de processus causant l'extinction des petites populations. Nous avons proposé une définition des effets Allee élémentaires générés par des processus génétiques. Des exemples d'effets Allee génétiques ont été identifiés dans la littérature. Certains diminuent le taux d'accroissement des populations (effet Allee démographique).

Le deuxième objectif est de rechercher l'existence d'un effet Allee génétique et éventuellement démographiques dans des populations expérimentales de *Venturia canescens*, un Hyménoptère parasitoïde avec sl-CSD. Au préalable, des marqueurs microsatellites ont été mis au point et utilisés pour montrer une relation négative entre diversité génétique et proportion de mâles diploïdes dans des populations naturelles et captives isolées ou goulotées. Nous avons également montré que la dépression de consanguinité autre que la production de mâles diploïdes est faible chez *V. canescens*. Les mâles diploïdes s'accouplent mais sont stériles. Nous avons créé des populations expérimentales de *V. canescens* avec différents niveaux de diversité génétique suivies sur plusieurs générations. Ces populations ont été élevées de façon à favoriser la mise en place d'une dynamique hôte-parasitoïde et donc de goulots d'étranglement récurrents. Un effet Allee génétique dû à la production de mâles diploïdes a été détecté mais il n'influençait pas le taux d'accroissement ni la probabilité d'extinction des populations. Les extinctions observées semblent surtout due à la stochasticité démographique.

Ce travail constitue une approche expérimentale visant à dissocier les rôles des processus démographiques et génétiques dans l'extinction des populations. La détermination des causes d'extinction des populations permet une meilleure efficacité des procédures de gestion des populations menacées, invasives ou d'auxiliaires de lutte biologique.

Mots-clés : petites populations, interactions génétique-démographie, vortex d'extinction, complementary sex determination, effets Allee génétiques, populations expérimentales

ABSTRACT

Genetic and demographic processes can drive small populations to extinction. Both categories of processes are often studied separately. However, their interaction can generate extinction vortices, as predicted for some Hymenoptera species. Hymenoptera are haplodiploids: males are haploid and females are diploid. In species with single-locus complementary sex determination (sl-CSD), hemizygous at the CSD develop in males and heterozygous diploids, in females. Homozygous develop in diploids males, which are often unviable or sterile. Theoretical studies suggest that the production of diploid males associated with demographic and environmental stochasticity may drive small populations into an extinction vortex.

The first objective of this work is to stimulate collaboration between genetics and demography by proposing a concept that includes both categories of processes affecting small populations. We proposed a definition for component Allee effects generated by genetic processes. Genetic Allee effects were detected in the literature and some of them lower population growth rate (demographic Allee effect).

The second objective was to investigate the presence of a genetic, and maybe demographic, Allee effect in experimental populations of the parasitoid *Venturia canescens*, a Hymenoptera with sl-CSD. Before that, microsatellite markers were developed and used to show a negative relationship between genetic diversity and proportion of diploid males in natural and captive isolated and bottlenecked populations. We have also shown that inbreeding depression other than the production of diploid males is negligible in *V. canescens*. Diploid males can mate but they are sterile. We created experimental populations of *V. canescens* with different levels of genetic diversity. Populations were monitored across several generations. They were reared so as to favour the apparition of a host-parasitoid dynamics, with thus recurrent bottlenecks. A genetic Allee effect due to the production of diploid males was detected but it did not influence the growth rate or the probability of extinction of populations. Extinction events observed thus seem mainly due to demographic stochasticity.

This work constitutes an experimental approach aiming at separating the roles of genetic and demographic processes in population extinction. Determination the causes of extinction enables the use of more efficient management strategies for threatened, invasive or biocontrol agent populations.

Keywords: small populations, genetic-demography interactions, extinction vortex, complementary sex determination, genetic Allee effects, experimental populations

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SOMMAIRE

LISTE DES FIGURES

1.1 Les causes d'extinction des petites populations

1.1.1 Causes extrinsèques et intrinsèques à la population

Il est depuis longtemps admis que les petites populations s'éteignent plus rapidement que les autres (Lande 1993; Shaffer 1981). L'identification des causes d'extinction des petites populations présente un intérêt théorique et constitue une question centrale pour trois disciplines en plein essor : la biologie de la conservation, la biologie de l'invasion et la lutte biologique.

La biologie de la conservation vise à documenter la biologie des populations, espèces et écosystèmes menacés dans le but de les préserver (Soulé 1985). L'émergence de cette discipline de gestion de crise répond à la prise de conscience progressive de la valeur économique et sociétale de la biodiversité, et de son érosion rapide. Les écosystèmes naturels fournissent de très nombreux services aux sociétés humaines : production de nourriture, formation des sols, pollinisation, élimination de déchets, beauté des paysages…etc. (Balmford *et al.* 2002; Chapin *et al.* 2000; Humphries *et al.* 1995). L'impact des activités humaines sur les écosystèmes naturels a fortement augmenté au cours du XX^{ème} siècle, au point que les taux d'extinction d'espèces sont actuellement 100 à 1000 fois plus élevés qu'avant l'apparition de l'humanité. Les changements d'usage des terres, les rejets de gaz à effet de serre et les introductions d'espèces font partie des nombreuses causes de déclin et d'extinction des populations et espèces naturelles (Butchart *et al.* 2010; Chapin *et al.* 2000). Identifier les causes du déclin d'une population est une étape nécessaire à l'élaboration de mesures de conservation efficaces. Ainsi, une étude approfondie du cycle de vie du papillon *Maculinea arion* dans une petite population en déclin a permis d'augmenter les effectifs suite à un rétablissement du pâturage dans les prairies où l'espèce est présente (Thomas *et al.* 2009). La mise en évidence de dépression de consanguinité dans une petite population isolée de vipère (Madsen *et al.* 1996) a mené à l'introduction temporaire de mâles issus d'une autre population, suivie d'une augmentation de la taille de la population et d'une réduction de la dépression de consanguinité dans la population.

Tout comme la biologie de la conservation, la biologie de l'invasion est une discipline récente apparue en réponse à une crise, celle de l'augmentation rapide du nombre de populations invasives, due au développement des échanges de biens et de personnes sur de longues distances. Les populations invasives sont des populations qui s'établissent dans une nouvelle aire géographique et y prolifèrent (Mack *et al.* 2000). Elles ont presque toujours des impacts négatifs

pour les populations natives et/ou les activités humaines. Ainsi, l'une des principales causes du déclin actuel de la biodiversité est l'introduction de populations invasives (Vitousek *et al.* 1997). Les populations invasives ont aussi un impact économique considérable, estimé à plus d'un milliard de dollars et concernant principalement l'agriculture, la pêche, les services écosystémiques et la santé humaine (Pimentel *et al.* 2001). Bien que le terme « invasive » évoque de grandes populations, la prolifération d'une espèce invasive est le résultat de l'établissement d'un petit nombre d'individus introduits dans un nouvel environnement. Ces petites populations ont une probabilité d'extinction élevée (Jeschke & Strayer 2005; Suarez *et al.* 2005; Williamson 2006) mais, parmi les nombreuses populations introduites, quelques-unes parviennent à survivre et à s'accroître dans leur nouvel environnement. L'un des objectifs de la biologie de l'invasion est de comprendre les causes de leur survie afin de limiter leur propagation et d'optimiser les stratégies de prévention et de lutte (Liebhold & Tobin 2008; Simberloff 2009; Tobin *et al.* 2011). L'étude des populations invasives du papillon *Lymantria dispar* a ainsi permis de détecter les seuils de taille de population en-dessous desquels les populations ont une forte probabilité de s'éteindre, même sans action d'éradication (Tobin *et al.* 2009).

Enfin, la lutte biologique classique est une invasion planifiée. Cette pratique de lutte contre les organismes nuisibles (ravageurs des cultures, adventices, parasites…) existe depuis plus d'un siècle et consiste à introduire un ennemi naturel (auxiliaire) d'un ravageur dans un nouvel environnement où le ravageur est présent. L'objectif est que l'auxiliaire s'établisse et contrôle durablement les populations du ravageur (Eilenberg *et al.* 2001). Le même procédé de capture en population naturelle, multiplication en laboratoire et réintroduction dans la nature est appliqué en biologie de la conservation, avec réintroduction dans la zone d'origine ou dans une autre zone (Sarrazin & Barbault 1996). Dans les deux cas, les populations introduites sont issues d'un petit nombre d'individus prélevés dans une population naturelle et multipliés en laboratoire avant d'être relâchés dans la zone cible. Pour favoriser l'établissement de la population, il est important de connaitre ses causes d'extinction potentielles (Bale *et al.* 2008). Cependant, les études recherchant les causes d'extinction des populations d'auxiliaires restent rares (mais voir Fauvergue & Hopper 2009 et Fauvergue *et al.* 2007). Des expériences en laboratoire ont toutefois montré que la probabilité d'extinction de populations de l'auxiliaire *Trichogramma chilonis* est influencée par la taille de la zone d'introduction et l'origine géographique des insectes (Vercken *et al.* 2013).

Quel que soit le contexte d'étude, les causes de l'extinction ou de la survie d'une population sont très souvent multiples. Dans un article marquant, Caughley (1994) distingue les causes d'extinction intrinsèques et extrinsèques à la population. Les causes intrinsèques à la population (« small-population paradigm ») sont des conséquences de la petite taille de la population. Les

causes extrinsèques (« declining-population paradigm ») sont les facteurs externes à la population qui réduisent sa taille. Les causes intrinsèques ont suscité un intérêt théorique plus important car leurs processus sont généralisables à de nombreuses espèces. Au contraire, les causes extrinsèques sont plus dépendantes du contexte, favorisant une approche plus empirique. Bien que ses implications pour la gestion des populations menacées aient été sujettes à débat (Asquith 2001; Hedrick 1996), la dichotomie cause extrinsèques/intrinsèques à la population est largement acceptée (Asquith 2001).

1.1.2 Causes extrinsèques à la population

Les causes d'extinction extrinsèques à la population diffèrent suivant que l'on considère des populations en déclin, étudiées en biologie de la conservation, ou des populations introduites, comme les populations invasives, les auxiliaires de lutte biologique, ou les réintroductions d'espèces menacées.

Le déclin d'une population est souvent dû à l'interaction de plusieurs facteurs (Caughley, 1994; Krebs, 2002). Les plus fréquents sont la surexploitation, la destruction d'habitats et l'introduction d'espèces exotiques. Dans un écosystème, l'extinction d'une population peut aussi entraîner celle d'autres populations avec lesquelles elle interagissait : prédateurs, pollinisateurs, symbiontes…etc (Diamond 1989). Ces causes sont le plus souvent d'origine anthropique mais peuvent aussi être générées par de catastrophes naturelles. Pour déterminer les causes du déclin d'une population, Caughley (1994) recommande une approche au cas par cas basée sur une bonne connaissance de la biologie de l'espèce étudiée. Ceci permet d'identifier les facteurs potentiellement impliqués dans le déclin. En mesurant les corrélations au cours du temps entre ces facteurs et la taille de la population, on peut ensuite sélectionner certains facteurs (*e.g.* Wolf & Mangel 2008). La dernière étape consiste à confirmer par l'expérience le rôle de ces facteurs dans le déclin de la population. Bien que cruciale, elle n'est pas réalisable pour toutes les populations et tous les facteurs de déclin, ce qui a conduit à la proposition de méthodes alternatives comme l'utilisation de modèles démographiques ou la comparaison de plusieurs populations (Norris 2004; Peery *et al.* 2004). Ces méthodes ont, par exemple, montré que le déclin de populations de guillemot marbré (*Brachyramphus marmoratus*) en Californie étaient dues à une quantité de nourriture trop faible et/ou, selon les années, à une forte prédation des nids.

Pour les populations introduites ou réintroduites, la cause de la petite taille de population est plus évidente. Ce sont de nouvelles populations fondées par un nombre restreint d'individus, les fondateurs, issus d'une population plus grande. Les populations réintroduites et les auxiliaires de lutte biologiques peuvent passer par une phase de multiplication en laboratoire avant d'être

introduits sur le terrain, mais leurs effectifs restent modestes comparés à ceux d'une population naturelle stable. Quand plusieurs événements d'introduction se succèdent, la probabilité de survie de la population dépend de la pression de propagule, déterminée par le nombre d'événements d'introduction et le nombre moyen d'individus introduits à chaque introduction, comme cela a été observé pour les populations invasives (Lockwood *et al.* 2005) et les auxiliaires de lutte biologique (Hopper & Roush 1993, Fauvergue *et al.*, 2012).

Toutes ces causes de déclin génèrent un goulot d'étranglement : une réduction importante de la taille de la population. Cette diminution peut être très rapide, comme dans le cas des populations introduites. Au fur et à mesure que la taille de la population diminue, certains processus ont un impact de plus en plus fort sur la démographie de la population, alors que leur effet est négligeable dans de grandes populations. Ces processus constituent les causes d'extinction intrinsèques à la population.

1.1.3 Causes intrinsèques à la population

Dans cette thèse, je m'intéresse aux causes d'extinction intrinsèques à la population. Ces processus sont traditionnellement divisés en deux catégories : les processus démographiques et génétiques. Les premières études sur les petites populations ont identifié des processus démographiques stochastiques (Athreya & Karlin 1971; Bartlett 1960) ou déterministes (Allee 1938; Odum 1953) pouvant réduire les effectifs des populations. Puis, les processus génétiques comme la dépression de consanguinité et la perte de variation génétique ont suscité un vif intérêt (Frankel 1974; Frankel & Soulé 1981; Obrien *et al.* 1983). L'importance accordée aux problèmes génétiques en biologie de la conservation appliquée a été critiquée, notamment par Lande (1988) qui a affirmé que la plupart des populations menacées s'éteignaient sous l'effet de processus démographiques avant que les processus génétiques n'aient un impact sur elles (voir aussi Boyce 1992; Caughley 1994). L'opposition entre processus génétiques et démographiques est renforcée par le fait que ces deux types de processus relèvent de disciplines différentes. Aujourd'hui, il est admis que les processus génétiques et démographiques peuvent influencer la survie des populations. Les paragraphes suivants présentent brièvement les principaux processus démographiques et génétiques qui influencent l'extinction des populations, en insistant sur les interactions entre démographie et génétique.

a) Processus démographiques

Parmi les processus démographiques, on distingue les processus stochastiques que sont la stochasticité démographique et la stochasticité environnementale et un processus déterministe : l'effet Allee.

Les populations sont caractérisées par des paramètres démographiques comme le nombre moyen de descendants par individu ou le sex ratio. La stochasticité démographique désigne les fluctuations aléatoires des paramètres démographiques dues à la taille finie des populations. Les valeurs des paramètres démographiques sont les moyennes de variables qui n'ont pas exactement la même valeur pour tous les individus dans la population. Le plus souvent, la valeur moyenne n'est pas réalisable par un individu car il s'agit d'un nombre décimal (*e.g.* 2,2 descendants par individu), alors que les nombres d'individus sont des nombres entiers (aucun individu n'aura 2,2 descendants). De plus, ces valeurs fluctuent aléatoirement suivant les individus. Une valeur extrême chez un individu aura un plus fort impact sur la valeur moyenne dans une petite population que dans une grande, où cette valeur extrême sera atténuée par celles des autres individus. La stochasticité démographique augmente donc la probabilité d'extinction des petites populations, en augmentant la variance des paramètres démographiques quand la taille de la population diminue (Lande 1988; May 1973). Il est ainsi plus probable qu'une petite population ne produise aucun descendant ou seulement des descendants du même sexe, ce qui entraînera son extinction. La stochasticité démographique peut être mesurée par le coefficient de variation de paramètres démographiques. Dans des populations expérimentales de drosophiles (*Drosophila birchii*), le coefficient de variation du taux d'accroissement était plus élevé dans les petites populations, qui avaient une probabilité d'extinction plus forte (Willi & Hoffmann 2009).

L'impact de la stochasticité démographique sur les petites populations peut être renforcé par la stochasticité environnementale. La stochasticité environnementale correspond à des fluctuations imprédictibles de la valeur moyenne des paramètres démographiques au cours du temps. Ces fluctuations peuvent avoir des causes abiotiques, comme le climat, ou biotiques, comme l'abondance des prédateurs ou des compétiteurs. L'impact de la stochasticité environnementale sur les valeurs moyennes des paramètres démographiques est indépendant de la taille de la population. Cependant, dans les petites populations, il augmente la probabilité que la population s'éteigne à cause de la stochasticité démographique, puisque les paramètres démographiques varient autour d'une moyenne plus faible (Drake 2004; Grevstad 1999b). La stochasticité environnementale semble avoir joué un rôle dans l'extinction de petites populations introduites d'une chrysomèle auxiliaire de lutte biologique car la variance du taux d'accroissement des populations était indépendante de la taille de la population (Grevstad 1999a).

L'effet Allee est un processus déterministe de densité-dépendance positive. Stephens *et al.* (1999) ont distingué les effets Allee élémentaires et les effets Allee démographiques. Un effet Allee élémentaire est une relation positive entre la taille ou la densité d'une population et une composante de la fitness individuelle (Figure 1.A), telle que le nombre de descendants, la survie juvénile ou la probabilité d'accouplement. Les composantes de la fitness influencent les paramètres démographiques affectés par la stochasticité démographique et environnementale (Jongejans *et al.* 2010). La combinaison des différentes composantes de la fitness détermine la fitness individuelle, dont la valeur moyenne dans une population peut être mesurée par le taux d'accroissement *per capita*. En parallèle des effets Allee, il existe des processus de densité-dépendance négative, comme la compétition, qui entraînent une augmentation d'une composante de la fitness quand la taille ou la densité d'une population diminue. Un effet Allee élémentaire sur une composante de fitness peut donc être compensé par un processus densité-dépendant sur une autre composante, ce qui fait que la fitness moyenne ne montre pas de relation positive avec la taille de la population. Quand cette compensation est insuffisante, l'effet Allee affecte la fitness moyenne des individus et on observe un effet Allee démographique : une relation positive entre la taille ou la densité d'une population et son taux d'accroissement *per capita*, qui est une mesure de la fitness individuelle (Figure 1.B). On parle d'effet Allee démographique faible quand le taux d'accroissement reste positif quelle que soit la taille de la population. Quand le taux d'accroissement devient négatif en-dessous d'une certaine taille de population (le seuil d'effet Allee), on parle d'effet Allee démographique fort (figure 1.B). Ce type d'effet Allee peut entraîner la population dans un vortex d'extinction : quand la taille d'une population passe en-dessous du seuil d'effet Allee, son taux d'accroissement devient négatif donc la taille de la population diminue encore, elle a un taux d'accroissement encore plus négatif et ainsi de suite jusqu'à l'extinction. De nombreux mécanismes génèrent des effets Allee élémentaires (Berec *et al.* 2007; Courchamp *et al.* 2008; Courchamp *et al.* 1999). Le plus connu d'entre eux est la difficulté à trouver un partenaire sexuel dans les petites populations. C'est, par exemple, le mécanisme supposé responsable de l'échec de l'établissement de populations introduites du copépode *Hesperodiaptomus shoshone* en-dessous d'une certaine densité (Kramer & Sarnelle 2008). La défense contre les prédateurs, les comportements de coopération, la modification de l'habitat sont d'autres exemples de mécanismes à l'origine d'effets Allee élémentaires.

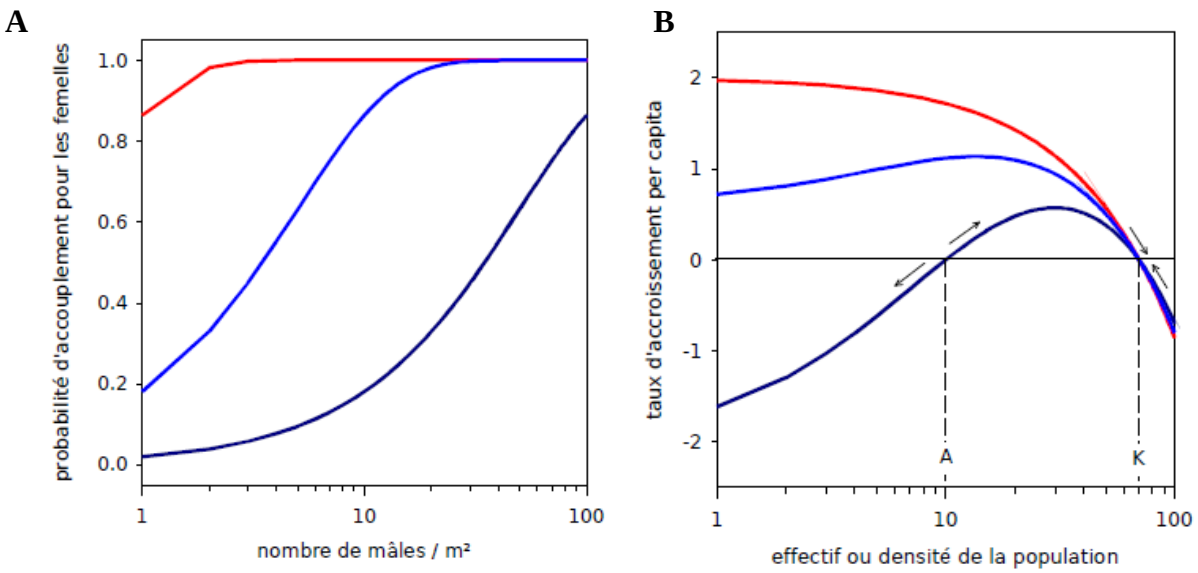

Figure 1 : Les effets Allee. **A**. Exemple d'effet Allee élémentaire : la probabilité d'accouplement des femelles augmente avec la densité des mâles dans la population. La courbe rouge représente une situation sans effet Allee, les deux courbe bleue, un effet Allee plus (bleu foncé) ou moins (bleu clair) fort. **B**. effet Allee démographique La courbe en rouge représente une population affectée par la densité-dépendance seule, la courbe en bleu clair, un effet Allee démographique faible et la courbe bleu foncé, un effet Allee démographique fort. A est le seuil d'effet Allee et K la capacité de charge. D'après {Fauvergue, 2009 #152}.

b) Processus génétiques

Dans les petites populations, la consanguinité et la dérive génétique peuvent avoir un fort impact sur la probabilité de persistance des populations.

Un individu est consanguin si ses parents possèdent au moins un ancêtre commun. Plus cet ancêtre commun est proche dans le temps et plus l'individu est consanguin. On parle de dépression de consanguinité quand les individus plus consanguins ont une fitness plus faible que les autres (Hedrick & Kalinowski 2000). La dépression de consanguinité est le plus souvent due à l'expression d'allèles délétères récessifs. La superdominance (meilleure fitness des hétérozygotes) et des interactions épistatiques (entre allèles de différents gènes) peuvent aussi entraîner de la dépression de consanguinité (Charlesworth & Willis 2009; Kristensen *et al.* 2009). Dans une petite population panmictique, la probabilité de s'accoupler par hasard avec un apparenté est plus élevée que dans une grande population (Malécot 1969). Les individus y sont donc, en moyenne, plus consanguins et plus affectés par la dépression de consanguinité, ce qui diminue la fitness moyenne dans la population et peut entrainer son extinction. Ceci a été démontré, entre autres, sur des

populations expérimentales de drosophiles (*Drosophila melanogaster* et *D. virilis*) et de souris (*Mus musculus*) maintenues à différents niveaux de consanguinité (Frankham 1995).

La dérive génétique désigne les variations aléatoires des fréquences alléliques au cours des générations dans une population. Ces variations sont dues à l'échantillonnage aléatoire des allèles lors de la méiose et de la fécondation dans des populations de taille finie. La dérive génétique est l'équivalent, au niveau génétique, de la stochasticité démographique. Tout comme la stochasticité démographique, la dérive génétique a un impact plus fort sur les petites populations, dont les fréquences alléliques fluctuent plus au cours du temps. Quand ces fluctuations aléatoires deviennent plus importantes que les variations de fréquences déterminées par la sélection, la dérive peut fixer des allèles délétères et éliminer des allèles bénéfiques, réduisant la fitness moyenne dans la population (Kimura 1983; Whitlock 2000). L'accumulation d'allèles délétères a ainsi entraîné l'extinction de populations expérimentales de levure *(Saccharomyces cerevisia*, Zeyl *et al.* 2001). La dérive génétique affecte aussi les gènes sous sélection balancée, comme les gènes de compatibilité (*e.g.* complexe majeur d'histocompatibilité, systèmes d'auto-incompatibilité des plantes) ou les gènes avec superdominance (*e.g.* anémie falciforme). Ce type de sélection maintien plusieurs allèles d'un même gène dans la population. La perte par dérive de diversité allélique à ces loci diminue la fitness moyenne dans les populations en augmentant la fréquence des accouplements incompatibles ou la production d'homozygotes, à la fitness faible que les hétérozygotes (Byers & Meagher 1992; Levin *et al.* 2009; Zayed & Packer 2005). Dans des petites populations d'une plante en danger d'extinction (*Rutidosis leptorrhynchoides*), une partie des individus, qui avaient reçu du pollen incompatible, avaient une fécondité nulle ou réduite (Young & Pickup 2010).

La dérive génétique diminue aussi le potentiel adaptatif des populations. Les populations présentant une diversité génétique faible ont une faible probabilité de posséder un allèle adapté à de nouvelles conditions environnementales. Elles sont donc plus susceptibles de s'éteindre suite à une modification de leur environnement (Pease *et al.* 1989). Frankham *et al.* (1999) ont montré que des populations de drosophiles (*Drosophila melanogaster*) ayant subi un fort goulot d'étranglement s'adaptaient moins bien à une augmentation de la concentration en sel dans leur milieu d'élevage.

c) Interaction entre processus démographiques et génétiques

Une population peut être affectée à la fois par des processus génétiques et démographiques (*e.g.* Burgman & Lamont 1992). Parfois, l'interaction entre ces deux types de processus peut entraîner la population dans un vortex d'extinction : un (ou des) processus génétique, comme la

dépression de consanguinité, diminuent la taille de la population, qui devient donc plus sensible à la stochasticité démographique et environnementale. La population peut alors devenir encore plus petite, ce qui va accentuer la dépression de consanguinité et ainsi de suite jusqu'à l'extinction de la population (Gilpin & Soulé 1986). Des modèles théoriques de vortex d'extinction ont été développés pour les principaux processus génétiques : dépression de consanguinité (Tanaka 2000), fixation de mutations délétères (fonte mutationnelle, Lynch *et al.* 1995), perte de potentiel adaptatif (Gomulkiewicz & Holt 1995), perte d'allèles de compatibilité (Kirchner *et al.* 2006). Par contre, la rétroaction entre processus génétiques et démographiques n'a jamais été démontrée expérimentalement. Toutefois, Palomares *et al.* (2012) ont montré la cooccurrence de processus génétiques (dépression de consanguinité) et démographiques (stochasticité démographique) dans une petite population de lynx. Les deux types de processus influencent la probabilité d'extinction de populations expérimentales de l'insecte *Bemisia tabacci*, dont l'effectif et le degré de consanguinité ont été manipulés (Hufbauer *et al.* 2013). Fagan & Holmes (2006) ont étudié la dynamique avant extinction de 10 populations de vertébrés. Ces dynamiques sont cohérentes avec l'hypothèse d'un vortex d'extinction mais les mécanismes à l'origine du vortex ne sont pas recherchés. Il peut donc s'agir de vortex d'extinction dus seulement à des processus démographiques, comme l'interaction entre effet Allee et stochasticité démographique ou environnementale (Dennis 2002), ou l'effet Allee seul (effet Allee fort, Wang & Kot 2001).

Peu d'études empiriques ont recherché une rétroaction entre processus génétiques et démographiques menant à l'extinction des populations, ou, plus simplement, l'existence des deux types de processus dans une même population. Cela peut être dû au fait que les deux types de processus sont étudiés par deux disciplines différentes – la dynamique et la génétique des populations – bien qu'ils aient des conséquences similaires pour les populations. Ainsi, certains processus génétiques créent une relation entre taille de population et fitness individuelle moyenne, tout comme les effets Allee (*e.g.* Fischer *et al.* 2003; Willi *et al.* 2005). De plus, le rôle des processus génétiques dans l'extinction des populations a longtemps été débattu. Certains auteurs ont avancé l'hypothèse que les populations s'éteignaient à cause de processus démographiques avant que les processus génétiques n'aient eu un impact sur elles (Boyce 1992; Lande 1988). Depuis, on a montré que les processus génétiques sont présents dans certaines populations en voie d'extinction, et agissent parfois sur quelques générations seulement (Amos & Balmford 2001; Frankham 2005; Spielman *et al.* 2004). Le rôle prédominant d'un type de processus dépend des caractéristiques de la population et de l'espèce concernées (Robert 2011). Les approches intégrant les processus démographiques et génétiques, y compris évolutifs (*e.g.* Turcotte *et al.* 2013), sont désormais encouragées (Kokko & Lopez-Sepulcre 2007; Pelletier *et al.* 2009; Robert *et al.* 2007).

1.2 Un cas d'interaction entre génétique et démographie chez les Hyménoptères

1.2.1 Le « single-locus Complementary Sex-Determination »

Le système de détermination du sexe de certains Hyménoptères peut être à l'origine d'un vortex d'extinction dû à une perte de diversité génétique à un locus sous sélection balancée. La plupart des Hyménoptères se reproduisent par parthénogénèse arrhénotoque : les mâles sont haploïdes issus d'œufs non fécondés, alors que les femelles sont diploïdes issues d'œufs fécondés. Au niveau moléculaire, il existe plusieurs systèmes de détermination du sexe chez les Hyménoptères. Les deux principaux sont l'empreinte génomique, que nous ne détaillerons pas ici, et le « Complementery Sex-Determination » abrégé en CSD (Heimpel & de Boer 2008). Le CSD est le système ancestral (Asplen *et al.* 2009; Schmieder *et al.* 2012), divisé en « single-locus CSD » (sl-CSD) et « multiple-locus CSD » (ml-CSD). Avec le sl-CSD, le sexe d'un individu dépend de sa composition allélique à un seul gène. S'il possède deux allèles différents à ce gène de détermination du sexe, il se développera en femelle. S'il possède un seul type d'allèles, il sera mâle. Sous ces conditions, il apparaît qu'une femelle est nécessairement diploïde et que tous les haploïdes sont des mâles (Figure 2.A). Il existe aussi une troisième catégorie d'individus : les diploïdes homozygotes au gène du CSD, qui vont se développer en mâles diploïdes le plus souvent stériles ou non viables. Les mâles diploïdes sont issus de parents possédant un allèle en commun au gène du CSD (Figure 2.B). Comme les mâles diploïdes sont produits à la place des femelles, leur production équivaut à une surmortalité femelle. Chez les espèces à ml-CSD, il existe plusieurs gènes de détermination et du sexe et seuls les individus homozygotes à tous ces gènes deviennent des mâles diploïdes. La production de mâles diploïdes est donc plus rare avec le ml-CSD.

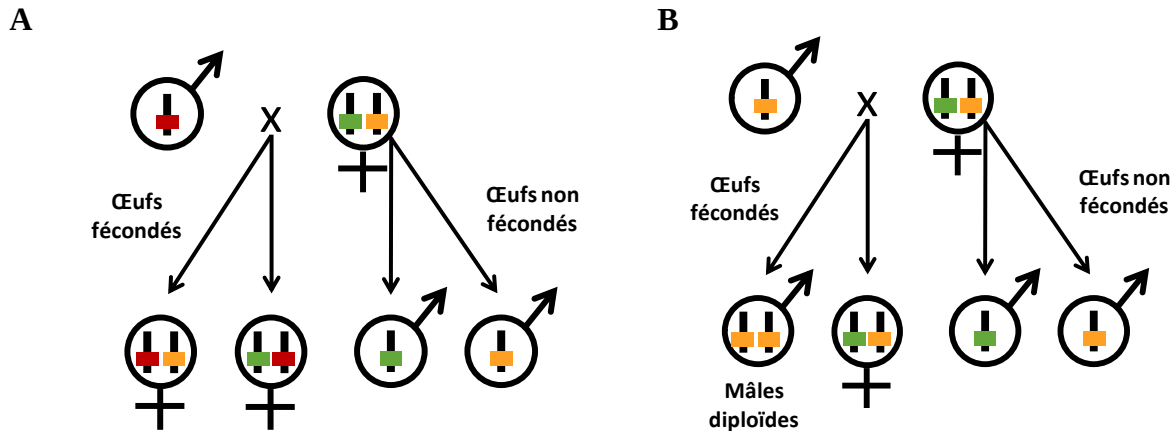

Figure 2 : Génotypes au gène du CSD des descendants de parents portant des allèles différents (**A**) ou portant un allèle en commun (**B**). Ce dernier cas engendre la production de mâles diploïdes.

Les bases moléculaires du CSD ne sont que partiellement connues. Le gène du CSD a d'abord été identifié chez l'abeille domestique *Apis mellifera* (Beye *et al.* 2003) et d'autres espèces d'abeilles, puis chez une dizaine d'autres espèces de l'infra-ordre de Aculéates (Schmieder *et al.* 2012). Chaque allèle code pour une protéine et la production de deux protéines différentes active le gène *fem* dont la transcription déclenche une cascade de gènes aboutissant à la féminisation de l'individu (Beye 2004).

La détermination du sexe par sl-CSD est suspectée pour plus de 80 espèces chez qui on a trouvé des mâles diploïdes, incluant des espèces d'importance économique (Harpur *et al.* 2013; van Wilgenburg *et al.* 2006) tels que l'abeille domestique, des pollinisateurs (*e.g. Bombus terrestris*), des auxiliaires de lutte biologique (*e.g. Cotesia rubecula*), des espèces invasives (*e.g. Solenopsis invicta*) et des tenthrèdes ravageuses des cultures (*e.g. Athalia rosae ruficornis*). Pour 25 de ces espèces, la détermination du sexe par sl-CSD a été confirmée par des croisements contrôlés. Deux exemples de ml-CSD ont été mis en évidence, chez les parasitoïdes *Cotesia vestalis* (de Boer *et al.* 2008) et *C. rubecula* (de Boer *et al.* 2012).

La présence de mâles diploïdes dans des populations naturelles a été documentée dans plus de 60 espèces (*e.g.* Cournault & Aron 2009; Souza *et al.* 2010). Ces proportions varient de l'absence de mâles diploïdes détectés (*Bombus distinguendus*, Charman *et al.*, 2010) à plus de 50% de mâles diploïdes parmi les mâles collectés pour certaines populations d'*Euglossa imperialis* (Zayed *et al.* 2004) ou des populations invasives de *Solenopsis invicta* (Krieger *et al.* 1999). Trois études ont aussi montré une relation négative entre taille de population et proportion de mâles diploïdes (Alves *et al.* 2011; Darvill *et al.* 2012; Ross *et al.* 1993). La grande majorité des espèces étudiées sont sociales (abeilles, bourdons, fourmis). Pour ces espèces, la proportion élevée de mâles diploïdes parmi les mâles dans certaines populations peut être due à un sex-ratio fortement biaisé en faveur des femelles, qui entraîne la production d'un grand nombre de mâles diploïdes si la reine est accouplée avec un mâle portant le même allèle qu'elle au gène du CSD. A ma connaissance, seules trois populations d'insectes solitaires ont été étudiées. Il s'agit de la guêpe solitaire *Ancistrocerus antilope* (Chapman & Stewart 1996) et de deux parasitoïdes du genre *Cotesia*. Les proportions de mâles diploïdes par population étaient de 25% pour *A. antilope*, 10% pour *Cotesia glomerata* (Ruf *et al.* 2013) et 15% pour *C. rubecula* (de Boer *et al.* 2012). Dans ce dernier cas, la population étudiée a été introduite comme auxiliaire de lutte biologique, ce qui pourrait expliquer le taux élevé de mâles diploïdes rencontré dans cette espèce à ml-CSD (Zayed *et al.* 2004).

A partir des proportions de mâles diploïdes dans une population, on peut estimer le nombre d'allèles au gène du CSD présents dans cette population. Dans une population panmictique avec un sex-ratio équilibré, le nombre d'allèles au sl-CSD est théoriquement égal à $1/d$, où d est la

proportion de mâles diploïdes parmi les individus diploïdes (Adams *et al.* 1977). Cette méthode de calcul appliquée à quelques populations naturelles fait état de 10 à 25 allèles par population (Adams *et al.* 1977; Antolin *et al.* 2003; Kukuk & May 1990; Ross & Fletcher 1985). Cette méthode est basée sur le fait que les allèles au gène du CSD sont sous sélection balancée : les allèles rares ont une probabilité plus faible de se retrouver à l'état homozygote et d'entrainer la production d'un mâle diploïde, qui ne peut pas transmettre ses gènes. Les allèles rares sont donc favorisés par la sélection balancée jusqu'à ce qu'ils atteignent une fréquence d'équilibre de $1/n$, où n est le nombre d'allèles au gène du CSD dans la population. Après quoi, leur fréquence fluctue autour de $1/n$ (Yokoyama & Nei 1979). Les séquences génétiques du gène *csd* identifié chez l'abeille *A. mellifera* présentent d'ailleurs des signatures de sélection balancée (Cho *et al.* 2006; Hasselmann *et al.* 2008). Des nombres d'haplotypes au gène du CSD peuvent donc être mesurés comme estimation du nombre d'allèles dans une population, sans que l'on sache cependant si chaque haplotype correspond à un allèle différent. Chez plusieurs espèces d'abeilles, le nombre d'haplotypes détectés est élevé, de l'ordre d'une douzaine dans un échantillon d'une cinquantaine d'individus (Liu *et al.* 2013; Liu *et al.* 2012; Wang *et al.* 2013; Wang *et al.* 2012).

Dans les petites populations, la production de mâles diploïdes peut entraîner l'apparition d'un vortex d'extinction.

1.2.2 Interaction entre démographique et génétique : le « diploid male vortex »

La production de mâles diploïdes peut avoir des conséquences négatives sur la démographie des populations, principalement parce que ces mâles sont produits à la place de femelles. Par ailleurs, la diminution de l'effectif d'une population peut réduire la diversité génétique au gène du CSD et donc favoriser la production de mâles diploïdes. Pour mieux comprendre ces interactions entre processus génétiques et démographiques, des modèles théoriques individu-centrés ont été élaborés. Quelques études de populations expérimentales ont aussi été réalisées.

a) Modèles théoriques

Le premier modèle explorant les relations entre taille de population et diversité génétique au gène du CSD a été élaboré en 1992 par Stouthamer *et al.* pour un contexte de lutte biologique classique. Il montre d'abord que les populations comportant un faible nombre d'allèles au gène du CSD ont un sex-ratio plus biaisé vers les mâles et un taux d'accroissement plus faible mais néanmoins positif dans les conditions choisies. Ces conséquences démographiques sont accentuées si les mâles diploïdes sont viables et s'accouplent. L'influence de deux paramètres de la lutte

biologique classique – le nombre d'individus prélevés et la taille de la population d'élevage – sur le nombre d'allèles au gène du CSD est alors explorée. Pour une population naturelle comportant 20 allèles au gène du CSD, il faut prélever un minimum de 20 à 30 femelles accouplées pour capturer presque tous les allèles. Au cours de la phase d'élevage, le nombre d'allèles diminue au cours du temps. Cette diminution est plus rapide dans les populations d'élevage de petite taille. Le nombre d'allèles est aussi négativement affecté par la stochasticité sur le nombre de descendants par femelle et le nombre d'accouplements par mâle. Bien que le modèle de Stouthamer *et al.* montre des interactions entre diversité génétique, sex ratio et taux d'accroissement des populations, il ne mentionne pas de vortex d'extinction, car les populations étudiées ont toutes un taux d'accroissement positif.

Le modèle proposé par Zayed & Packer (2005) est le premier à mentionner un vortex d'extinction dû à la production de mâles diploïdes. Il considère trois scénarios : (i) pas de sl-CSD, (ii) sl-CSD, les mâles diploïdes sont non viables et (iii) sl-CSD, les mâles diploïdes s'accouplent et produisent des filles triploïdes stériles. Pour chaque scénario, des populations caractérisées par leur capacité de charge et leur nombre moyen de descendants par femelle sont simulées sur 100 générations. Le nombre initial d'allèles au gène du CSD dans une population est déterminé par sa capacité de charge. Les populations qui ont une capacité de charge faible et/ou un faible nombre de descendants par femelle ont une probabilité et une vitesse d'extinction plus élevées. Les probabilités et vitesse d'extinction sont beaucoup plus élevées dans les scénarios avec sl-CSD que dans celui sans sl-CSD, ce qui indique que la stochasticité démographique n'est pas la principale cause d'extinction des petites populations avec sl-CSD. Les populations où les mâles diploïdes s'accouplent ont une probabilité d'extinction plus élevée, du fait de la production de femelles non viables par les mâles diploïdes. Le nombre d'allèles au gène du CSD décroît au cours du temps et le sex-ratio à la dernière génération avant extinction est très biaisé vers les mâles, avec plus de la moitié des populations comportant exclusivement des mâles à la génération avant l'extinction.

Les auteurs expliquent ces résultats par l'apparition d'un vortex d'extinction du au sl-CSD (Figure 3). Dans les petites populations, le faible nombre d'allèles au gène du CSD entraîne la production de mâles diploïdes. Comme ils sont produits à la place des femelles, ces dernières sont moins nombreuses, et le taux d'accroissement de la population est réduit. S'il devient négatif, la taille de la population diminue, ce qui peut encore réduire le nombre d'allèles au gène du CSD et ainsi de suite jusqu'à l'extinction. Ce modèle implique donc une rétroaction entre processus génétiques et démographiques.

Après la mise en évidence du « diploid male vortex », Hein *et al.* (2009) ont testé l'influence de plusieurs paramètres démographiques et comportementaux sur la probabilité de survie des

populations avec sl-CSD dans un modèle en métapopulations. Le nombre de sous-populations présentes après 5000 générations est utilisé comme mesure de la viabilité de la population. Les auteurs démontrent que les extinctions de populations sont plus fréquentes quand : (i) les mâles diploïdes sont viables et s'accouplent, (ii) les mâles diploïdes qui s'accouplent sont stériles ou ont une fertilité moindre que celle des mâles haploïdes, (iii) la probabilité de dispersion des individus est faible, (iv) le sex-ratio est biaisé vers les mâles, (v) le nombre moyen de descendants par individu est faible, (vi) la probabilité que les femelles acceptent l'accouplement avec des mâles diploïdes est élevée et (vii) le nombre maximal d'allèles au gène du CSD est faible.

Cette étude met l'accent sur les comportements qui limitent la production de mâles diploïdes. La présence de comportements de dispersion ou d'évitement des accouplements avec des mâles diploïdes pourrait expliquer la persistance du sl-CSD comme système de détermination du sexe, malgré le coût imposé par la production de mâles diploïdes (van Wilgenburg *et al.* 2006).

A ce jour, le « diploid male vortex » n'a pas été démontré expérimentalement, même si quelques études expérimentales ont abordé les conséquences démographiques de la production de mâles diploïdes.

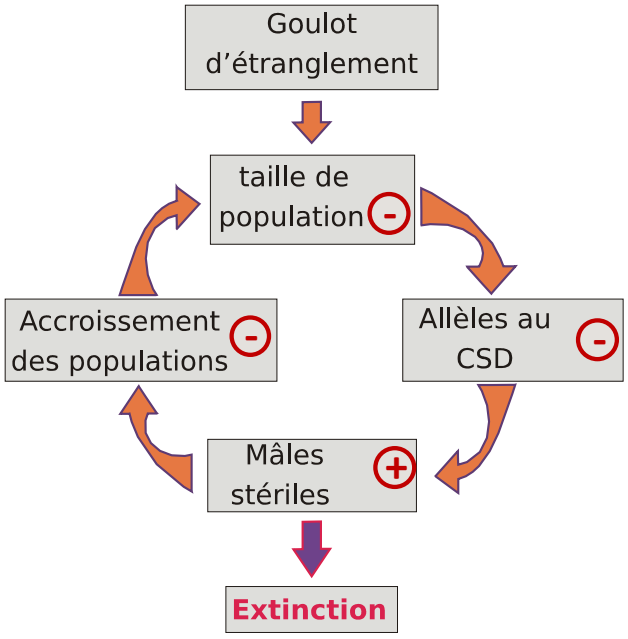

Figure 3 : Le « diploid male vortex ». Suite à un goulot d'étranglement, le nombre d'allèles au gène du CSD diminue, ce qui augmente la production de mâles diploïdes et réduit le nombre de femelles. Le taux d'accroissement est donc plus faible qu'à la génération précédente. Si le taux d'accroissement devient négatif, la taille de la population diminue et la population entre dans un vortex d'extinction. D'après Zayed & Packer (2005).

b) Etude de populations expérimentales

La fondation de populations expérimentales semble nécessaire pour démontrer en toute rigueur l'existence d'un vortex d'extinction. Elle permet de manipuler indépendamment l'effectif et la diversité génétique des populations, afin de mettre en évidence l'impact de ces facteurs sur la production de mâles diploïdes et les paramètres démographiques des populations (nombre de femelles, taux d'accroissement, etc). De plus, toutes les populations peuvent être élevées dans les mêmes conditions environnementales, ce qui limite les potentiels effets confondants de l'environnement. Quatre études ont utilisé des populations expérimentales pour mesurer l'impact démographique de la production de mâles diploïdes. Trois d'entre elles portent sur des espèces sociales, et la dernière étude concerne un parasitoïde.

Les Hyménoptères sociaux vivent en colonies fondées par une ou plusieurs reines, qui sont les individus reproducteurs. La reine pond en grande majorité des œufs fécondés qui donneront des ouvrières non reproductrices chargées de l'approvisionnement et de l'entretien de la colonie. Si la reine s'est accouplée avec un mâle portant le même allèle qu'elle au CSD, une partie de ses œufs destinés à être des ouvrières seront en fait des mâles stériles, ce qui réduit la force de travail disponible pour la colonie. On s'attend donc à ce que les colonies comportant des mâles diploïdes soient moins performantes que les autres, en particulier chez les espèces monoandre, pour lesquelles la moitié des œufs fécondés se développeront en mâles diploïdes. Cette hypothèse a été testée et confirmée pour trois espèces : la fourmi invasive *Solenopsis invicta*, l'abeille domestique *Apis mellifera*, et le bourdon *Bombus terrestris*.

S. invicta est une espèce monoandre dont les colonies peuvent comporter une ou plusieurs reines. En laboratoire, la présence dans une colonie d'une reine produisant des mâles diploïdes réduit le nombre d'ouvrières et la survie de la colonie. Sur le terrain, les colonies comportant une seule reine produisant des mâles diploïdes sont absentes, ce qui semble indiquer qu'elles ne sont pas capables de survivre dans des conditions naturelles (Ross & Fletcher 1986). Chez l'abeille domestique, les colonies comportent une seule reine accouplée avec plusieurs mâles. En fonction des génotypes de ses partenaires au sl-CSD, la reine produit une descendance diploïde composée de 0 à 50% de mâles diploïdes. Pour obtenir des colonies avec différentes proportions de mâles diploïdes, Tarpy & Page (2002) ont artificiellement inséminé des reines avec le sperme de trois de leurs frères, la reine ayant une chance sur deux de porter le même allèle que son frère. L'utilisation de frères pour tous les accouplements permet également d'exclure les différences entre colonies qui seraient dues à d'autres formes de dépression de consanguinité que la production de mâles diploïdes. Les colonies fondées par les reines ont été élevées en extérieur. La proportion de mâles diploïdes a affecté la survie hivernale des colonies : sur les 31 colonies de l'expérience, celles

comportant moins de 28% de mâles diploïdes ont toutes survécu à l'hiver alors que seules 37,5% des autres colonies ont survécu. Une expérience similaire a été réalisée avec des reines de *B. terrestris* accouplées soit avec un mâle non apparenté, soit avec un frère. La moitié des reines accouplées avec un frère produisent des mâles diploïdes, qui constituent 50% de leur descendance diploïde. Les colonies comportant des mâles diploïdes ont produit moins de descendants et avaient un taux de croissance en laboratoire et une probabilité de survie sur le terrain plus faibles (Whitehorn *et al.* 2009). Ces trois expériences montrent un fort impact de la production de mâles diploïdes sur la taille et la survie des colonies. Cependant, les études s'arrêtent au niveau de la colonie, qui représente la descendance d'une seule femelle, et ne sont jamais conduites au niveau de la population, probablement en raison des difficultés matérielles que présentent la fondation et le suivi de populations expérimentales d'insectes sociaux.

La dernière étude de populations expérimentales concerne un parasitoïde, *Cotesia glomerata*, dont les mâles diploïdes sont partiellement fertiles. On peut tout de même s'attendre à ce que la production de mâles diploïde affecte négativement les populations car les mâles diploïdes sont produits à la place des femelles, qui sont donc moins nombreuses dans les populations à faible diversité génétique. Les populations expérimentales ont été réparties en trois niveaux de diversité génétique : 1, 3 ou 6 familles fondatrices. Douze populations par modalité ont été fondées avec le même nombre d'individus (10) et un sex-ratio équilibré. A chaque génération, le même nombre d'hôtes (60) était fourni à chaque population. Il n'a été observé aucun effet de la diversité génétique initiale sur les traits d'histoire de vie mesurés (nombre d'hôtes parasités, taille de population, taux d'émergence, durée de développement, sex-ratio, taille du corps) ni sur la survie des populations. En revanche, les valeurs de la plupart des traits d'histoire de vie ont diminué au cours du temps, et la majorité des populations s'est éteinte au cours des 10 générations de l'expérience. La démographie des populations de *C. glomerata* semble donc peu affectée par la production de mâles diploïdes mais plus sensible à d'autres formes de dépression de consanguinité.

En résumé, les trois études expérimentales du « diploid male vortex » sur des espèces sociales s'intéressent aux colonies et non à la population toutes entière et la seule étude portant sur une espèce non sociale ne montre pas d'impact démographique de la production de mâles diploïdes. On peut regretter que cette étude de populations de parasitoïdes occulte un élément important de la biologie de ces organismes : l'interaction entre les dynamiques de population des parasitoïdes et de leurs hôtes entraîne souvent des fluctuations d'effectifs des deux populations, de sorte qu'elles peuvent passer par des goulots d'étranglement. C'est l'une des raisons pour lesquelles nous avons choisi des populations d'Hyménoptères parasitoïdes pour tester le « diploid male vortex ».

1.3 Intérêt des Hyménoptères parasitoïdes pour tester le « diploid male vortex »

1.3.1 Les Hyménoptères parasitoïdes

Les parasitoïdes sont des organismes dont l'adulte mène une vie libre, alors que la larve se développe aux dépends d'un hôte dont elle entraîne sa mort en résultat de son développement (Eggleton & Gaston 1990; Godfray 1994). La majorité des insectes parasitoïdes sont des Hyménoptères et ont pour hôtes d'autres insectes. On distingue les endoparasitoïdes, dont la larve se développe à l'intérieur de l'hôte, et les ectoparasitoïdes qui s'alimentent sur la surface externe de l'hôte. Certains parasitoïdes sont solitaires – une seule larve par hôte – et d'autres grégaires – une femelle pond plusieurs larves par hôte (Godfray 1994). Les parasitoïdes jouent un rôle écologique important en régulant les populations d'insectes phytophages, ce qui a abouti à leur utilisation en lutte biologique contre les ravageurs des cultures (LaSalle & Gauld 1991). Chez les Hyménoptères parasitoïdes, deux familles, les Ichneumonidés et les Braconidés, comportent des espèces dont le sexe est déterminé par « complementary sex determination » (Asplen *et al.* 2009). Les petites populations de ces espèces sont donc susceptibles d'être affectées par le « diploid male vortex ».

1.3.2 La dynamique hôte-parasitoïde

Le mode de vie des parasitoïdes est intermédiaire entre ceux des prédateurs et des parasites. Comme les prédateurs, les parasitoïdes tuent leurs hôtes mais la mort de l'hôte intervient dans un certain délai après l'attaque. Entre la ponte de l'œuf de parasitoïde et la mort de l'hôte, la larve de parasitoïde se développe en parasite. Le fait que le parasitoïde tue l'hôte permet une régulation des populations d'hôtes par les populations de parasitoïdes, générant une dynamique cyclique pour les deux populations, proche d'une dynamique proie-prédateur (Figure 4). Cette dynamique a fait l'objet de nombreuses études théoriques prenant en compte la répartition spatiale des individus (*e.g.* Hassell & May 1973), les interactions avec d'autres espèces d'hôtes ou de parasitoïdes (*e.g.* Comins & Hassell 1979) ou le comportement de recherche des parasitoïdes (*e.g.* Bernstein *et al.* 1988). L'élevage de populations d'hôtes et de parasitoïdes en microcosmes en laboratoire permet de confronter les prédictions des modèles à des données expérimentales (*e.g.* Bonsall & Hastings 2004).

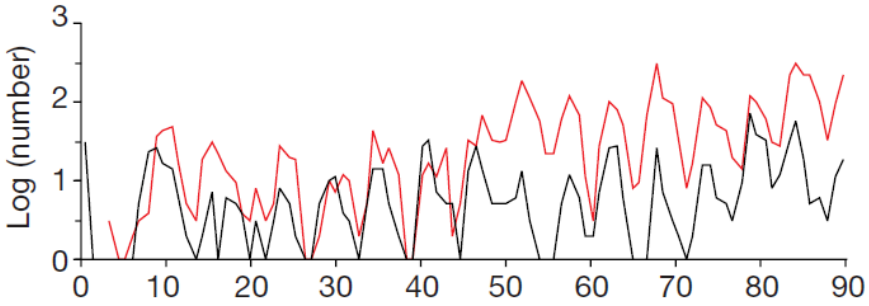

Figure 4 : Exemple de dynamique hôte-parasitoïde dans une population du parasitoïde *Venturia canescens* (courbe rouge) élevée en laboratoire sur l'hôte *Plodia interpunctella* (courbe noire). D'après Bjornstad *et al.* (2001).

La dynamique hôte-parasitoïde génère des goulots d'étranglement récurrents dans la population de parasitoïdes. L'utilisation de parasitoïdes en lutte biologique classique implique aussi un goulot d'étranglement lors de la capture d'individus dans leur aire native. Pour les espèces de parasitoïdes avec CSD, ces goulots d'étranglement peuvent réduire la diversité génétique au gène du CSD, augmenter la production de mâles diploïdes et entraîner la population dans le « diploid male vortex ». Cependant, si les populations subissent régulièrement des goulots d'étranglement, certaines espèces peuvent avoir développé des comportements adaptatifs permettant la survie des populations tels que la dispersion postnatale (Mazzi *et al.* 2011), l'évitements des partenaires apparentés (Metzger *et al.* 2010) ou portant le même allèle au gène du CSD (Thiel *et al.* 2013) ou la polyandrie (Crozier & Page 1985).

A.Bompard, I. Amat, X. Fauvergue et T. Spataro (Données non publiées) ont élaboré un modèle simulant une population de parasitoïdes avec CSD en interaction avec une population d'hôtes. Les trois scénarios présentés par Zayed & Packer (2005) ont été utilisés : populations sans CSD, populations avec CSD et mâles diploïdes non viables, populations avec CSD et mâles diploïdes viables, qui s'accouplent mais sont stériles. Les populations avec CSD et mâles diploïdes non viables ont une probabilité d'extinction plus faible que les populations sans CSD (Figure 5). Le CSD réduit l'amplitude des cycles d'environ 25%. Les populations subissent donc des goulots d'étranglement moins forts et sont moins affectées par la stochasticité démographique. La viabilité et l'accouplement des mâles diploïdes augmentent la probabilité d'extinction par rapport aux populations avec mâles diploïdes non viables (Figure 5). En effet, si toutes les femelles d'une population sont accouplées avec des mâles diploïdes, qui sont stériles, la population s'éteint car aucune femelle n'est produite à la génération suivante. Dans les populations avec CSD, initiées avec 25 allèles au gène du CSD, la plupart des allèles sont perdus au cours des premières générations et les populations persistent ensuite avec 2 à 5 allèles, ce qui suggère que la diversité génétique initiale

a peu d'impact sur la probabilité de survie des populations. Les précédents modèles qui ont étudié les conséquences populationnelles de la production de mâles diploïdes considéraient des populations avec capacité de charge constante et montraient que le CSD réduisait la probabilité de survie des populations (Hein *et al.* 2009; Stouthamer *et al.* 1992; Zayed & Packer 2005). Le modèle de Bompard *et al.* montre qu'il n'en est pas de même pour une population de parasitoïde en interaction avec celle de son hôte. Dans cette situation, le CSD augmente la probabilité de survie des populations (si les mâles diploïdes sont non viables) ou modifie la plage de paramètres où la population persiste (si les mâles diploïdes sont viables et stériles). Ces prédictions n'ont cependant jamais été testées expérimentalement.

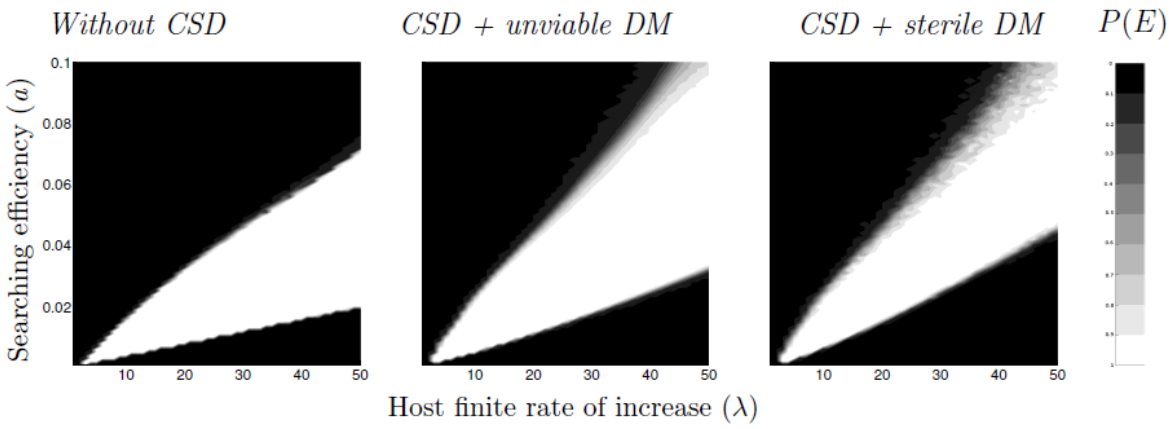

Figure 5 : Probabilité d'extinction (*P(E)*) de la population de parasitoïdes en fonction du taux d'accroissement intrinsèque de l'hôte (λ) et de l'efficacité de recherche du parasitoïde (*a*) qui est une mesure de la capacité des parasitoïdes à détecter les hôtes. Les résultats sont présentés pour des populations sans CSD, avec CSD et mâles diploïdes non viables et avec CSD et mâles diploïdes viables et stériles.

1.3.3 Le modèle biologique *Venturia canescens*

Nous avons utilisé le parasitoïde *Venturia canescens* Gravenhorst (Hymenoptera : Ichneumonidae) pour tester le « diploid male vortex » dans une population de parasitoïdes en interaction avec celle de son hôte. *V. canescens* (Figure 6) est un endoparasitoïde solitaire de larves de Lépidoptères, principalement des Pyralidés, vivant dans les fruits secs ou les denrées stockées [Salt, 1976 #164]. Il existe deux sous-espèces, l'une asexuée et l'autre sexuée. La forme asexuée se reproduit par parthénogénèse thélytoque et est donc exclusivement constituée de femelles. On la trouve principalement dans les milieux anthropiques tels que les minoteries ou les entrepôts de denrées stockées. La forme sexuée comprend des mâles et des femelles qui se reproduisent par parthénogénèse arrhénotoque et vivent en milieu naturel (Beukeboom *et al.* 1999; Schneider *et al.* 2002). Cette espèce, et surtout la forme asexuée, est élevée en laboratoire depuis des dizaines

d'années et a été utilisée lors d'expériences d'écologie comportementale (*e.g.* Desouhant *et al.* 2005; Driessen & Bernstein 1999), évolution des traits d'histoire de vie (*e.g.* Harvey *et al.* 2001; Roberts & Schmidt 2004), génétique (*e.g.* Beukeboom & Pijnacker 2000; Hellers *et al.* 1996), physiologie (*e.g.* Casas *et al.* 2003; Pelosse *et al.* 2007) et dynamique des populations (*e.g.* Begon *et al.* 1995; Bonsall & Hassell 1998). Récemment, quelques études ont été menées sur le terrain. Avec des femelles thélytoques d'élevages, Desouhant *et al.* ont étudié la dispersion (2005) et la détection des hôtes et des sources de nourriture (2003) en milieu naturel. Quelques observations ont également été menées sur le taux de parasitisme (Driessen & Bernstein 1999), les stratégies d'accouplements (Metzger *et al.* 2008) et la structure génétique dans des populations naturelles arrhénotoques (Mateo Leach *et al.* 2012; Schneider *et al.* 2003). Chez *V. canescens*, le sexe est déterminé par sl-CSD et les mâles diploïdes sont entièrement viables (Beukeboom 2001). Il s'agit du scénario prédit comme le plus néfaste pour les petites populations, que ce soit avec une capacité de charge constante (Zayed & Packer, 2005) ou sous une dynamique hôte-parasitoïde (A.Bompard, I. Amat, X. Fauvergue & T. Spataro, données non publiées). Cette caractéristique, couplée à une bonne connaissance de l'espèce font de *V. canescens* un modèle biologique approprié pour tester le « diploid male vortex ».

Figure 6 : Une femelle de *V. canescens* (photo X. Fauvergue)

1.4 Plan de la thèse

La thèse s'articule autour de deux objectifs principaux. Le premier est de proposer un concept qui englobe des processus génétiques et démographiques menant à l'extinction des petites populations. L'effet Allee est actuellement un concept principalement démographique. Pourtant, certains processus génétiques, comme la dépression de consanguinité, ont des conséquences similaires à celles d'un effet Allee élémentaire : ils entraînent une baisse d'une composante de la

fitness quand la taille de la population diminue. Pour faire de l'effet Allee un concept fédérateur, reliant génétique et démographie, nous avons proposé une définition des effets Allee génétiques, recherché des exemples d'effets Allee génétiques dans la littérature et suggéré des méthodes de détection des effets Allee génétiques (Chapitre 2, Article I).

Le deuxième objectif est de tester expérimentalement la présence d'un effet Allee génétique dû à la production de mâles diploïdes chez *V. canescens*. Selon les modèles théoriques, les petites populations possèdent moins d'allèles au gène du CSD et produisent donc plus de mâles diploïdes que les grandes populations. Les mâles diploïdes étant produits à la place des femelles et le plus souvent stériles, les mères produisant des mâles diploïdes ont un nombre de descendants fertiles réduit par rapport à celles qui ne produisent pas de mâles diploïdes. Il s'agit donc d'un effet Allee élémentaire : quand la taille de population diminue, le nombre de descendants fertiles par femelle diminue. Cet effet Allee élémentaire entraîne un effet Allee démographique s'il réduit le taux d'accroissement de la population, comme prédit par les modèles théoriques. Si cet effet Allee démographique est fort, la population entre dans le « diploid male vortex » et s'éteint.

Pour répondre à cet objectif, nous avons d'abord cherché à savoir si un effet Allee élémentaire dû à la production de mâles diploïdes pouvait apparaître dans des populations naturelles ou des populations captives qui n'ont pas été créées spécifiquement pour le détecter. Pour cela, nous avons mesuré la ploïdie de mâles capturés dans des populations naturelles et captives ayant subi des goulots d'étranglement plus ou moins forts (Chapitre 3, Article II). Auparavant, nous avions besoin d'une méthode de mesure de la ploïdie des mâles, que nous utiliserons aussi dans les études suivantes. Nous avons développé des marqueurs génétiques qui permettent à la fois de mesurer la ploïdie des mâles et des paramètres de génétique des populations tels que la diversité génétique ou la structure des populations (Chapitre 3, Article II).

Nous avons ensuite recherché la présence d'un effet Allee élémentaire et d'un effet Allee démographique dus à la production de mâles diploïdes dans des populations expérimentales de *V. canescens*. Pour préparer cette expérience, nous avons mesuré le niveau de dépression de consanguinité chez les femelles, afin de tenir compte du rôle éventuel de la dépression de consanguinité autre que la production de mâles diploïdes dans l'apparition d'un effet Allee démographique (Chapitre 4, Article III). Nous avons également mesuré la fitness des mâles diploïdes (Chapitre 4, Article IV) pour affiner les prédictions et l'interprétation des résultats de l'expérience suivante. Enfin, nous avons créé des populations expérimentales de *V. canescens* avec différents niveaux de diversité génétique. Ces populations ont été élevées en interaction avec celles de leur hôte afin de générer une dynamique hôte-parasitoïde et des goulots d'étranglement récurrents. Les populations ont été suivies sur plusieurs générations afin de mesurer leur production

de mâles diploïdes, pour détecter un effet Allee élémentaire, et leur taux d'accroissement, pour détecter un effet Allee démographique (Chapitre 5, Article V).

La thèse comporte également en annexe un article qui présente un travail antérieur (stage de master) que j'ai terminé pendant la durée de ma thèse avec des membres de mon laboratoire d'accueil (Article VI).

CHAPITRE 2 : LES EFFETS ALLEE GENETIQUES

Les petites populations sont affectées par des processus génétiques et démographiques qui peuvent entraîner leur extinction. Ces processus sont souvent étudiés séparément, par les généticiens des populations d'un côté et les dynamiciens des populations de l'autre. Une approche englobant les deux types de processus améliorerait la compréhension de la biologie des petites populations (Kokko & Lopez-Sepulcre 2007; Pelletier *et al.* 2009). Le premier objectif de la thèse est de proposer un concept qui regroupe les processus génétiques et démographiques, afin de contribuer au dialogue entre génétique et démographie. Nous avons choisi de promouvoir l'intégration de processus génétiques parmi les mécanismes générant des effets Allee. Les mécanismes étudiés jusqu'à présent sont principalement écologiques (rencontre entre partenaires, défense contre les prédateurs, modification de l'environnement… etc) et, bien que des mécanismes génétiques soient parfois mentionnés (Courchamp *et al.* 2008), l'effet Allee génétique n'a jamais été défini.

L'effet Allee génétique est un effet Allee élémentaire. Dans la définition que nous proposons, deux conditions doivent être remplies pour qu'un effet Allee génétique apparaisse. La première est qu'une baisse de la taille de la population modifie sa structure génétique, *i.e.* les nombres, fréquences et identités des différents génotypes présents dans la population. La deuxième condition est que cette modification de la structure génétique entraine une baisse d'une composante de la fitness. Quand les deux conditions sont remplies, une baisse de la taille de la population entraine baisse d'une composante de la fitness, ce qui correspond à un effet Allee élémentaire. Nous avons identifié trois mécanismes génétiques qui peuvent générer des effets Allee. Ils représentent trois forces évolutives majeures : la consanguinité, la dérive et la migration. La consanguinité diminue l'hétérozygotie et favorise donc l'apparition de dépression de consanguinité qui affecte négativement certaines composantes de la fitness. Dans les petites populations, la dérive peut être plus forte que la sélection. Quand elle surpasse la sélection négative et positive, elle mène à la fixation d'allèles délétères et à la perte des allèles bénéfiques, ce qui diminue une ou plusieurs composantes de la fitness (fardeau de dérive). Quand la dérive surpasse la sélection balancée, la diversité au locus concerné diminue, et la composante de fitness affectée par ce locus est réduite. Enfin, dans une petite population, l'arrivée de migrants représente une plus grande proportion de la population totale après migration que dans une grande population. En cas de dépression hybride ou de maladaptation des migrants, les petites populations ont des valeurs plus faibles pour certaines composantes de la fitness (fardeau de migration). Nous avons trouvé dans la littérature 15 exemples

qui démontrent un effet Allee génétique. Chaque mécanisme est illustré. Des effets Allee génétiques sont aussi suspectés dans une quarantaine d'autres études. A partir de ces exemples, nous proposons des méthodes de détection des effets Allee génétiques en recommandant la fondation de populations expérimentales dans lesquelles on manipule la structure génétique (*via* le nombre et l'apparentement des fondateurs par exemple) indépendamment de la taille de la population.

Nous avons trouvé seulement deux exemples d'effets Allee génétiques entraînant un effet Allee démographique dû à la dépression de consanguinité dans des populations naturelles (Saccheri *et al.* 1998) ou à la dépression de consanguinité et/ou au fardeau de dérive dans des populations expérimentales (Newman & Pilson 1997). Cependant, d'autres études, sans rechercher d'effet Allee élémentaire, montrent un effet Allee démographique qui pourrait être d'origine génétique.

Les effets Allee génétiques diffèrent des effets Allee écologiques en ce qu'ils impliquent deux conditions, qu'il importe de vérifier car l'une peut avoir lieu sans l'autre. Quand une population est affectée par un effet Allee génétique, il existe un délai entre le changement de taille de la population et ses conséquences pour la fitness. Ce délai est absent dans le cas d'un effet Allee écologique. Certaines populations subissent des effets Allee génétiques et écologiques. Des mécanismes écologiques peuvent aussi influencer des mécanismes génétiques et inversement. Ceci souligne l'importance d'étudier les aspects génétiques et démographiques dans les petites populations.

Le deuxième objectif de la thèse est de tester la présence d'un effet Allee génétique du au système de détermination du sexe chez *V. canescens*. Les chapitres suivants y sont consacrés. On s'attend à observer un effet Allee génétique dont la première condition est une relation positive entre la taille de la population et son nombre d'allèles au gène du CSD. Sous la deuxième condition, les populations qui ont moins d'allèles au CSD produisent moins de descendants fertiles par femelle car des mâles diploïdes, généralement stériles, sont produits à la place de femelles. Cet effet Allee élémentaire peut entraîner un effet Allee démographique : le taux d'accroissement diminue avec la taille de la population. S'il s'agit d'un effet Allee démographique fort, la population est entraînée dans le « diploid male vortex » (Zayed & Packer, 2005).

Article I

The demography and genetics of small populations meet under the genetic Allee effect

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Introduction

The Allee effect – a decrease in fitness caused by a decrease in population size – jeopardizes the persistence of small populations, be they declining (threatened species; Courchamp et al. 1999; Gascoigne et al. 2009) or bottlenecked (introduced/invasive species; Taylor and Hastings 2005; Tobin et al. 2011). Unveiled by the American ecologist Warder Clyde Allee in the 1930s, this concept has received growing interest from population ecologists over the last two decades. In 1999, Stephens *et al.* formalized the definition of Allee effects with a crucial distinction between **component** and **demographic Allee effects** (Stephens and Sutherland 1999; Stephens et al. 1999), and two years later, Wang and Kot (2001) highlighted the nuance between **strong** and **weak demographic Allee effects**. In parallel, Allee effects have been discovered in a wide range of taxa, with various underpinning mechanisms: mate-finding, predator dilution, habitat amelioration, and various cooperative behaviors (Courchamp et al. 2008; Courchamp et al. 1999). Several component Allee effects can occur in the same population (Berec et al. 2007), sometimes driving a demographic Allee effect (Angulo et al. 2007; Berec et al. 2007).

So far, most studies of Allee effects have considered average values for the **components of fitness** under scrutiny and have therefore ignored the variability of genotypes within populations. However, fitness components are also under the influence of the population **genetic structure**, which depends on population size (Leimu et al. 2006). Genetic mechanisms may thus generate component Allee effects. There is indeed accumulating evidence that genetic processes influence population dynamics, and it is now well admitted that inbreeding depression (Frankham 1995; Spielman et al. 2004), loss of genetic variation (Amos and Balmford 2001; Gomulkiewicz and Holt 1995), and accumulation of deleterious mutations (Lande 1994; Lynch et al. 1995) can lower population growth rate and even drive small populations to extinction. Genetic processes can impact populations at an ecological time scale of only few generations (Glémin 2003; Spielman et al. 2004), especially when combined with demographic processes such as demographic and environmental stochasticity (Hanski and Saccheri 2006; Tanaka 2000).

In contrast with the pervasive evidence that genetic mechanisms affect the dynamics of small populations, less than ten publications have referred to a genetic Allee effect after describing a positive relationship between population size and fitness underpinned by genetic mechanisms. There are two putative explanations for the paucity of literature on the genetic Allee effect. First, the genetic Allee effect has never been clearly defined, so that referring to a genetic Allee effect may not appear strongly convincing. Second, genetic mechanisms occurring in small populations are a classic ground for population genetics whereas the Allee effect is a concept primarily derived from population dynamics. The dialogue between the two disciplines may have been too slight yet

(Kokko and Lopez-Sepulcre 2007; Metcalf and Pavard 2007; Pelletier et al. 2009) for the genetic Allee effect to raise as a robust federative paradigm.

The aim of the present work is to propose the genetic Allee effect as a heuristic framework to approach interplays between genetics and demography in small populations. We first propose a formal definition for the genetic Allee effect and describe potential underpinning processes. We review evidence for genetic Allee effects, including population genetic studies, which, although not referring to the Allee effect, provide numerous examples of genetic Allee effects. From these examples, we then propose methods to detect genetic Allee effects (Box). The last section of the paper discusses the specificity, limits and demographic consequences of genetic Allee effects.

Glossary

Census population size: Number of individuals in a population. Census population size may be independent from population density (number of individuals per surface unit).

Component Allee effect: A decrease in the value of any component of individual fitness caused by a decrease in population size or density (Stephens et al. 1999). Recently defined as a depensatory feedback of population size or density on a single demographic rate (*sensu* Herrando-Perez et al. 2012). A component Allee effect may, or may not, cause a demographic Allee effect, depending on the integration of positive and negative density-dependent forces acting on the different components of fitness.

Component of fitness: Any vital rate occurring at different stages of the life history of an organism and contributing positively to population growth rate (*e.g.* fecundity, juvenile survival, mating probability...). The different components of fitness combine to produce total fitness. Total fitness can be defined as a measurable feature of alleles, genotypes or traits, which predicts their numerical representation in future generations (Hunt and Hodgson 2010). Average total fitness in a population is usually assessed by the per capita growth rate.

Demographic Allee effect (or ensemble Allee effect, *sensu* Herrando-Perez et al. 2012): A decrease in the per capita growth rate of the population caused by a decrease in population size or density. A demographic Allee effect is necessarily caused by one or several component Allee effects (Berec et al. 2007; Stephens et al. 1999).

Epistasis: The influence of an allele, at one locus, on one or several other alleles at other loci.

Fst: A measure of the genetic differentiation between two populations, often calculated from genetic polymorphism at neutral loci.

Genetic structure: The number, frequencies and identities of the different genotypes present in a population.

Heterosis: The higher fitness of a hybrid offspring compared to the fitness of any of the parents.

Inbreeding coefficient: The probability that an individual is homozygous at a given locus because it carries two copies of the same ancestral allele.

Negative frequency-dependent selection: A situation in which individuals carrying rare alleles have a higher fitness than individuals carrying more common alleles (Charlesworth 2006).

Overdominance: The higher fitness of heterozygous individuals at a locus compared to homozygous individuals.

Qst: A measure of the genetic differentiation of a quantitative trait between two populations.

Underdominance: The lower fitness of heterozygous individuals at a locus compared to homozygous individuals.

Weak/strong demographic Allee effect: A weak demographic Allee effect occurs when the per capita population growth rate remains positive even at smallest population size or density. A strong demographic Allee effect occurs when the per capita population growth rate becomes negative below a threshold population size or density. This "Allee threshold" (Wang and Kot 2001) is an unstable equilibrium: any population declining below the Allee threshold is deterministically doomed to extinction.

Definition

One fundamental principle of evolutionary biology is that a population is a collection of different genotypes bore by a number of different individuals. Changes in population genetic structure yield changes in the number and/or frequencies of some genotypes, and in turn, changes in the fitness components that these genotypes influence (Soulé 1980). Hence, the population genetic structure is expected to affect the fitness of individuals. These relations underlie the Allee effect because one important determinant of population genetic structure is population size (Crow and Kimura 1970).

On this basis, we define a genetic Allee effect as "a decrease in the value of any fitness component caused by a change in population genetic structure resulting from a decrease in population size". The genetic Allee effect is thus a component Allee effect that requires two distinct conditions: (1) a decrease in population size causes a change in the population genetic structure, and (2), this change in the population genetic structure causes a decrease in the value of a component of fitness (Fig. 1). Population size, genetic structure, and component of fitness are the three cornerstones of the genetic Allee effect. Because density is not supposed to influence the genetic structure of a population, we exclude it from the definition of genetic Allee effects, which thus considers **census population size** only. The first condition of the genetic Allee effect is that population size impacts at least one of the following aspects of genetic structure: heterozygosity, frequency of beneficial or detrimental alleles and allelic richness (Fig. 1). Under the second condition, this change in the genetic structure of the population causes a decrease of a component of individual fitness. If (and only if) both conditions are realized, a genetic Allee effect is observed.

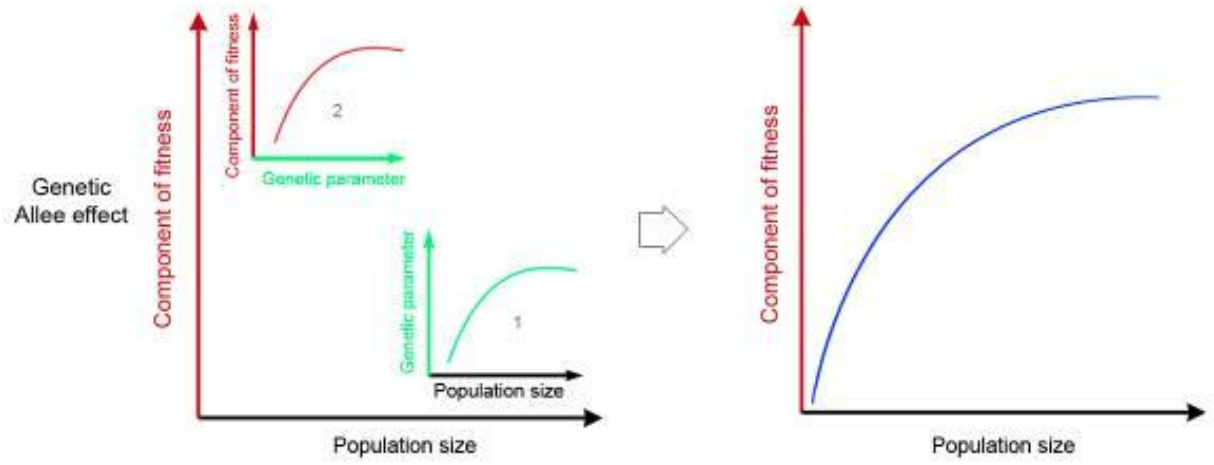

Figure 1. Two conditions are necessary for a genetic Allee effect to occur. Condition 1: A decrease in population size causes a change in a parameter of genetic structure (heterozygosity, frequency of detrimental and beneficial alleles, allelic richness). Condition 2: The change in genetic structure causes a decrease in the value of a component of fitness through inbreeding depression drift load or migration load. When both conditions occur in a population, a component genetic Allee effect is observed.

Mechanisms underlying genetic Allee effects

Our literature search (Annex) identified 15 studies showing strong evidence for one or several genetic Allee effects in natural or experimental populations (Table 1). We also found about 40 additional studies suggesting the occurrence of genetic Allee effects (see for instance the literature cited in Leimu et al. 2006). A thorough analysis of these published studies allowed delineating three different types of genetic Allee effects, each involving a major evolutionary force – inbreeding, drift and migration.

Inbreeding depression

As a population declines, the number of distinct families fatally decreases, and consequently, inbreeding becomes more frequent (Fig. 2, Malécot 1969). An inbred individual results from a cross between two genetically related individuals and is characterized by its **inbreeding coefficient**. Inbred individuals have fewer heterozygous loci than outbred individuals, so that heterozygosity decreases in declining populations (Frankham 1996, 1998). A widespread consequence of low heterozygosity is inbreeding depression, defined as the lower fitness of inbred compared to outbred individuals. Hence, inbreeding and inbreeding depression fulfill the two conditions defining the genetic Allee effect: (1) a decrease in population size causes a change in population genetic structure (heterozygosity) and (2), this change causes a decrease in one or several components of fitness (Fig. 2, Frankham 1998; Reed and Frankham 2003; Spielman et al. 2004; Wright 1977). The most common cause of inbreeding depression is the expression of deleterious recessive alleles brought at the homozygous state by inbreeding (Charlesworth and Willis 2009). Other causes imply **overdominance** and **epistasis**. For example, the buttercup, *Ranunculus reptans,* suffers from a genetic Allee effect due to inbreeding depression (Table 1.a, Willi et al. 2005). Small populations have a higher mean inbreeding coefficient (condition 1) and populations with higher inbreeding coefficient have lower seed production (condition 2).

Drift load

Genetic drift is a consequence of finite population size, defined as a change in allelic frequencies due to the random sampling of alleles from one generation to the next (Wright 1931). Like other random processes (*e.g.*, demographic stochasticity), the manifestations of genetic drift appear in small populations, with a decrease in allelic richness and heterozygosity (Fig. 2, Frankham 2005; Kimura and Crow 1964; Oostermeijer et al. 2003; Wright 1969).

Table 1: Published evidence for genetic Allee effects in natural and experimental populations. Genetic Allee effects in natural populations are classified according to their mechanism. Evidence for condition 1 indicates whether a relationship in the expected direction was tested and detected between population size and a parameter of genetic structure. Evidence for condition 2 indicates whether a relationship in the expected direction was tested and detected between a parameter of genetic structure and a component of fitness. Evidence for Allee effect indicates whether a positive relationship between population size and fitness component was observed. Experimental populations are populations in which genetic structure was manipulated independently of population size. For the three examples concerning experimental populations, the mechanism causing the genetic Allee effect was not identified.

Taxon	Evidence for condition 1	Parameter of genetic structure	Evidence for condition 2	Fitness component	Evidence for Allee effect	Reference
Inbreeding depression						
Plant *Cochealaria bavarica*	yes	Number of polymorphic loci and observed heterozygosity for 8 allozymes	untested	Fruit set	yes	Fischer *et al.*, 2003
Plant *Ranunculus reptans*	yes	Mean kinship coefficient*	yes	Clonal performance**; seed production	no	Willi et al., 2005
Insect *Melitaea cinxia*	yes	Observed heterozygosity at 7 enzyme and 1 microsatellite loci	yes	Larval survival; adult longevity; egg hatching rate	untested	Saccheri *et al.*, 1998
Drift load						
Plant *Ranunculus reptans*	yes	Allelic diversity at 7 allozymes	yes	Seed production	no	Willi et al., 2005
Plant *Hypericum cumulicola*	yes	Effective population size calculated with 10 microsatellite loci	untested	Cumulative fitness***	yes	Oackley *et al.*, 2012
Insect *Melitaea cinxia*	yes	Observed and expected heterozygosity and allelic richness at 9 microsatellite loci	untested	Mating rate; egg clutch size; hatching rate; larval survival; larval weight; larval group size at diapause; lifetime larval production	yes	Mattila *et al.*, 2012
Mollusc *Physa acuta*	yes	Gene diversity at 10 microsatellite loci	untested	Reproductive life-span	yes	Escobar *et al.*, 2008

Amphibian *Rana temporaria*	yes	Difference Fst-Qst for larval body size; allelic richness at 7 microsatellite loci	yes	Body size; larval survival	untested	Johansson *et al*., 2007
Plant *Cochealaria bavarica*	yes	Number of alleles per locus, number of polymorphic loci and observed heterozygosity for isoenzymes	untested	Compatibility of crosses	yes	Fischer et al., 2003
Plant *Ranunculus reptans*	yes	Allelic diversity at 7 allozymes	yes	Compatibility of crosses	no	Willi et al., 2005
Plant *Brassica insularis*	yes	Number and frequencies of alleles of compatibility	untested	Proportion of flowers pollinated with compatible pollen; fruit set	yes	Glémin *et al.*, 2008
Plant *Rutidosis leptorrhynchoides*	yes	Number and frequencies of alleles of compatibility	untested	Seed set	yes	Young & Pickup, 2010
Plant *Primula vulgaris*	yes	Morph frequencies	untested	Number of fruits per flower	yes	Brys *et al*., 2007
Migration load						
Plant *Eucalyptus aggregata*	yes	Hybridization rate	yes	Germination rate; survivorship	no	Field *et al*., 2008
Experimental populations						
Plant *Clarkia pulchella*	-	Number of founding families	-	Germination rate; survival rate from flower to fruit	yes	Newman & Pilson, 1997
Plant *Raphanus sativus*	-	Relatedness of founders	-	Fruit set; number of seeds per fruit	yes	Elam et al., 2007
Plant *Lolium multiflorum*	-	Number and relatedness of founders	-	Seed set; proportion of florets producing seeds	yes	Firestone et al., 2013
Insect *Bemisia tabaci*	-	Relatedness of founders	-	Number of offspring	yes	Hufbauer et al., 2013

*smaller populations have a lower allelic diversity at allozyme loci, and populations with lower allelic diversity have higher kinship coefficient, but the relationship between population size and kinship coefficient was not tested

**proportion of ovules producing seedlings × number of rooted rosettes

***proportion fruit set × seed number per fruit × proportion germinating × proportion surviving and reproducing × fecundity

Drift balances with selection to determine allelic richness. In contrast with drift, selection is a deterministic process whereby the frequency of an allele (or a genotype) varies across generations according to its effect on fitness: beneficial alleles increase in frequency while deleterious alleles decrease in frequency. The sign and strength of selection associated with a genotype can be defined as the positive or negative impact of this genotype on individual fitness. As such, it is independent of population size (Fig. 2) – we do not consider density-dependent selection. At small population size, changes in number and frequencies of alleles are mainly due to drift and not to selection (Kimura 1983; Whitlock 2000).

The change in the selection/drift balance at small population size modifies the allelic richness and heterozygosity of populations, driving the first condition of the genetic Allee effect (Fig. 2). The precise nature of the change in genetic structure depends on the type of selection at work. When drift overwhelms negative selection, mildly deleterious alleles increase in frequencies (Lanfear et al. 2013). They sometimes become fixed and constitute the drift load (Glémin 2003; Whitlock 2000). The corollary is the loss or decrease in frequency of beneficial alleles, which are under positive selection at large population sizes (Kimura and Crow 1964; Lanfear et al. 2013). In turn, the value of fitness components decreases if these components are influenced by loci that fix detrimental alleles and lose beneficial ones (Condition 2). Variations in larval body size in the frog *Rana temporaria* provide an interesting example, with drift stronger than selection in small populations and weaker than selection in large populations (condition 1). Consequently, small populations have a higher drift load on larval body size, and display lower values for this fitness component (Table 1.b, Johansson et al. 2007). Balancing selection also results in drift load. Balancing selection maintains several alleles at the locus under selection. The two main kinds of balancing selection are **overdominance** and **negative frequency-dependent selection**. In small populations where drift overwhelms balancing selection, rare alleles can be lost (Byers and Meagher 1992; Levin et al. 2009; Zayed and Packer 2005) and allelic frequencies move away from their optimum (condition 1), subsequently lowering the fitness components they influence (condition 2). For instance, plant self-incompatibility loci undergo balancing selection triggering a genetic Allee effect. This has been shown in the rare plant *Brassica insularis* where smaller populations have fewer alleles at the compatibility locus and thus lower fruit set (Glémin et al. 2008).

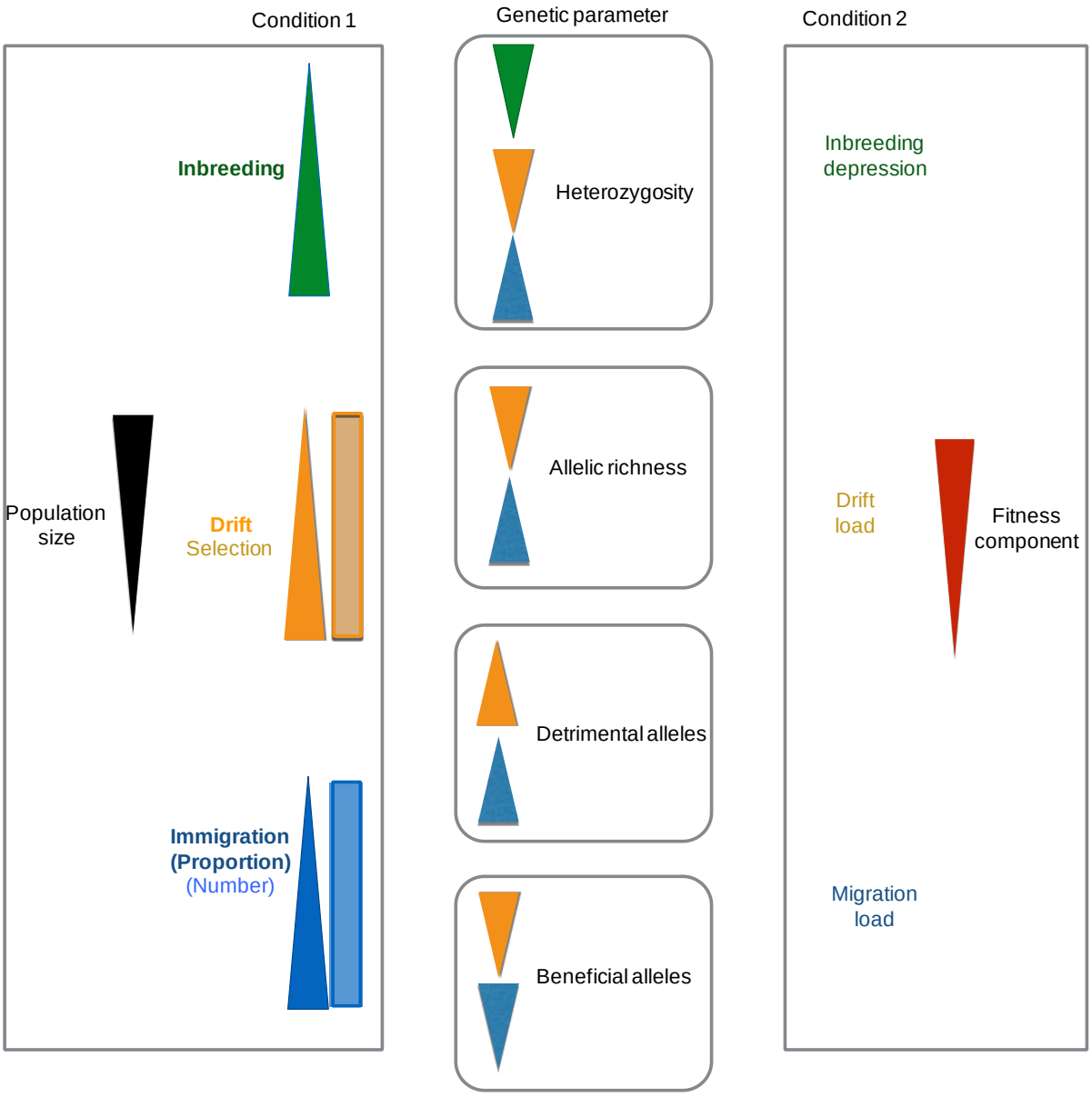

Figure 2. Scenarios that can lead to a genetic Allee effect. A decrease in population size causes an increase in inbreeding, a change in the drift/selection balance in favor of drift, or a higher proportion of immigrants in the population. These mechanisms impact the genetic structure of the population: heterozygosity, allelic richness, frequencies of detrimental and beneficial alleles (condition 1). Changes in genetic structure lower components of fitness through inbreeding depression, drift load or migration load.

Migration load

With effective dispersal, immigrants from a source population can bring new alleles into a sink population. Because the proportion of new alleles in the gene pool is higher in small sink populations than in large ones (Fig. 2), the change in genetic structure depends on population size, which fulfills the first condition of the genetic Allee effect (Fig. 1). A migration load can occur as a result of local maladaptation and/or outbreeding depression (Lenormand 2002; Ronce and Kirkpatrick 2001) caused by **underdominance** or deleterious epistatic interactions (Edmands 2002). As a consequence of overrepresentation of new alleles in small populations, the migration load is higher in small populations, which brings the second condition of the genetic Allee effect (Fig. 2). We found a single example of genetic Allee effect due to migration load (Table 1.d). Populations of *Eucalyptus aggregata* can hybridize with close species of eucalypts; hybridization rate increases with decreasing population size, and germination rate and seedling survivorship are lower in populations with a higher proportion of hybrids (Field et al. 2008).

Box: How to detect a genetic Allee effect

Detecting a genetic Allee effect requires investigations on each of the two conditions: (i) a decrease in population size causes a change in genetic structure, and (ii) this change in genetic structure causes a decrease in a component of fitness. To demonstrate a causal relationship for condition 2, two confounding effects must be avoided: environmental effects and ecological Allee effects. Controlling for environmental effects can be achieved by using constant environments in the experimental design (e.g. Oakley and Winn 2012) or by extracting fitness responses to environmental variability before analyzing the effect of population genetic structure (e.g. Saccheri et al. 1998). To untangle genetic from ecological Allee effects, an efficient method is to manipulate genetic structure experimentally via the number of founding families, while maintaining census size constant (e.g. Elam et al. 2007; Hufbauer et al. 2013). In this case, the number of founding families induces a change in a genetic parameter such as the frequencies of beneficial alleles (condition 1) that will then impact on a component of fitness such as the number of seeds (condition 2).

Different mechanisms can underlie the second condition including inbreeding depression, drift load, and migration load. Inbreeding depression occurs when individuals with higher inbreeding coefficients have lower fitness, a relation that can be assessed by measuring individuals from populations with known inbreeding coefficients (Willi et al. 2005) or offspring from controlled inbred and outbred crosses (Fischer et al. 2003; Oostermeijer et al. 2003).

Drift load is unveiled when between-population crosses result in **heterosis** and if this effect is higher in populations with lower heterozygosity or allelic richness (Escobar et al. 2008; Oakley and Winn 2012). Under certain conditions (Table I), the respective strengths of drift and selection can be estimated by measuring **Fst** and **Qst** between pairs of populations. Equal estimates of Fst and Qst for a quantitative fitness component suggests that drift is stronger than selection for this component. A positive relationship between the value of this fitness component and the Qst-Fst difference may indicate a relation between population genetic structure and fitness (condition 2; (Johansson et al. 2007). In the particular case of drift load involving plant self-incompatibility and balancing selection, the role of self-incompatibility in the relation between genetic structure and fitness can be approached via the proportion of incompatible crosses for which plants received pollen but produced no seeds (Fischer et al. 2003; Glémin et al. 2008; Willi et al. 2005).

Lastly, showing that immigrants and/or hybrids have lower fitness components than residents indicates an effect of migration load.

Table I. For each mechanism underpinning a genetic Allee effect, some specific parameters of the population genetic structure that can be measured and related to population size (condition 1) and components of fitness (condition 2).

Mechanism	Parameter of genetic structure	Relation with population size	Relation with fitness
inbreeding depression	inbreeding coefficient		
	heterozygosity		
drift load	difference Fst-Qst*		
	heterozygosity or allelic richness at neutral loci		
	number of compatibility alleles		
migration load	proportion of immigrant alleles		

***** Under certain conditions: (i) comparison of several groups of populations, with similar population size within groups and different population sizes between groups. Fst and Qst are calculated between all pairs of populations within groups. (ii) The component of fitness measured is a quantitative trait: it has a continuous distribution and is influenced by multiple loci with small effects.

Can genetic Allee effects cause demographic Allee effects?

Just like any other component Allee effect, genetic Allee effect can lower average individual fitness in small populations, and hence, yield a decrease in the population growth rate (Angulo et al. 2007; Lennartsson 2002). A notorious example is provided by research on the Glanville fritillary butterfly, *Melitaea cinxia*. In this species, small subpopulations have a lower heterozygosity than large ones, and less heterozygous females produce larvae with reduced survival (Saccheri et al. 1998). The Glanville fritillary is therefore subject to a genetic Allee effect. Controlled crosses in the laboratory and in the field confirmed the existence of inbreeding depression in this species (Nieminen et al. 2001). Further, inbreeding depression combined with poor resource availability causes the extinction of small subpopulations of *M. cinxia*, as shown by a higher probability of extinction of smaller and less heterozygous populations living in less favorable environmental conditions (Saccheri et al. 1998). In the plant *Clarkia pulchella*, a genetic Allee effect also impacts demography. Using an experimental approach, Newman and Pilson (1997) showed that populations with a lower number of founders are subject to inbreeding depression and/or drift load and consequently, have a lower growth rate. In several other species, genetically eroded populations were shown to have a lower growth rate (Fauvergue and Hopper 2009; Markert et al. 2010; Turcotte et al. 2013; Vercken et al. 2013; Wennersten et al. 2012). Although fitness components were not measured in these studies, the observed dynamics may reflect genetic Allee effects.

Comparison with ecological Allee effects

Temporal issues

Genetic Allee effects differ from ecological Allee effects on several grounds, one being the temporal scale. Genetic Allee effects are characterized by a time lag between a change in population size and its consequences on individual fitness whereas an ecological Allee effect may occur as soon as population size decreases (e.g., mating success decreases conjointly with decreased density). In the case of a genetic Allee effect, a decline in population size can change the genetic structure over tenths or thousands of generations, and fitness consequences may appear only several generations after this change (Amos and Balmford 2001; but see Hufbauer et al. 2013). Conversely, if population size increases, ecological Allee effects should fade almost immediately, whereas genetic Allee effects will not. For instance, after several generations of genetic drift, a population will not recover its initial level of genetic variation unless new alleles arise by mutation or migration. This implies that a population can be rescued from a demographic threat, but unless the underlying genetic structure is also restored, it may still suffer from a genetic Allee effect and be extinction-prone (what has been referred to as an extinction debt; Vercken et al. 2013).

Two conditions for the genetic Allee effect

Genetic Allee effects are idiosyncratic for their two conditions: each condition can occur independently of the other, but evidence for the concurrent occurrence of the two is necessary to demonstrate a genetic Allee effect. The first condition, a change in genetic structure caused by a decrease in population size can occur but yield no decrease in fitness (condition 1 does not imply condition 2). For instance, inbred individuals do not necessarily suffer from inbreeding depression; counter-intuitively, inbreeding can even result in the purge of deleterious alleles and an increase in fitness. Similarly, drift can randomly eliminate detrimental alleles and fix beneficial ones. Finally, immigration in small populations can lower the negative impact of drift and inbreeding by bringing new adapted alleles and increasing heterozygosity. The second condition, whereby a change in the genetic structure of a population triggers a decrease in a component of fitness, is not necessarily induced by the first condition, because genetic structures can change independently of population size under the influences of mutation, migration, or mating systems.

Evolutionary consequences

For their effects on individual fitness, ecological Allee effects could act as a selective force, and drive the evolution of adaptations to mitigate the sensitivity to population size or density (Courchamp et al. 2008). For instance, long-range volatile sex pheromones may prevent mating failures (Fauvergue et al. 2007). The same reasoning stands for genetic Allee effect as well. Inbreeding may have shaped the evolution of dispersal, inbreeding avoidance and self-incompatibility systems (Penn and Potts 1999; Perrin and Mazalov 2000).

Beyond a simple genetic Allee effect

As found for ecological Allee effects (Berec et al. 2007), several genetic Allee effects can co-occur in the same population. Small populations of buttercups (*Ranunuculus reptans*) were affected by inbreeding depression, drift load and loss of compatibility alleles (Willi et al. 2005). Genetic Allee effects can also combine with ecological Allee effects, as shown in experimental populations of the self-incompatible plants *Raphanus sativus* and *Lolium multiflorum* (Elam et al. 2007; Firestone and Jasieniuk 2013).

Some ecological Allee effects can also have genetic consequences. In plants, the reduction of pollinator density due to small population size generates a higher selfing rate and consequently, more inbreeding depression in small populations (Oostermeijer et al. 1998; Raijmann et al. 1994; Rusterholz and Baur 2010). This situation is not a genetic Allee effect because population size does

not directly impacts genetic structure. Conversely, genetic Allee effects have ecological consequences. The expression of detrimental or maladapted alleles can alter the ability of individuals to find a mate, escape predators, or modify their environment.

Moreover, some previously described ecological Allee effects could in fact reveal as genetic Allee effects: for instance, mating failures in small populations can result from a shortage of genetically compatible mates rather than a decreased encounter rate with conspecifics (Amos et al. 2001; Kokko and Rankin 2006; Moller and Legendre 2001). It is only via experimental manipulations of population size and genetic structure with factorial designs that genetic and ecological underpinnings of Allee effects will be disentangled.

Concluding remarks

Although they have been understudied so far, genetic Allee effects are important and ubiquitous. We propose a definition of the genetic Allee effect based on two necessary conditions: a decrease in population size changes the population genetic structure, which in turn causes a decrease in a component of individual fitness. Various mechanisms can generate the first condition: genetic drift, inbreeding, and migration. These mechanisms may trigger mechanisms that induce the second condition: inbreeding depression, drift load, and migration load. We found 15 demonstrations of genetic Allee effects in the literature, but they would probably be much more numerous with a wider use of methods to identify and distinguish genetic and ecological Allee effects occurring in the same populations. Our review also contributes to a more general perspective: integrating genetic mechanisms in the framework of the Allee effect provides a more comprehensive approach of the biology of small populations, and calls for a better dialogue between genetics and demography.

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Annex: Literature search

The literature search was conducted on ISI Web of Knowledge, on July 11th, 2013. We first looked for publications using the term "genetic Allee effect*". Then, we looked for publications that describe the two conditions of the genetic Allee effect without using this term. For this, we defined three sets of keywords, for words linked to population size, genetic structure or mechanisms of the condition 2 (Fig. 1), and components of fitness (Table I). We searched for publications mentioning at least one word from each set of keywords.

Table I: List of keywords used for the literature search.

Population size	**Genetic composition or condition 2 mechanisms**		**Fitness component**
"population size*"	"inbreeding coefficient*"		fitness
"size* of population*"	"coefficient* of inbreeding"		reprod*
"small population*"	"kinship coefficient*"		survival
"propagule pressure"	"coefficient* of relatedness"		lifespan
"experimental population*"	"genetic diversity"		longevit*
	"genetic varia*"		"life-history trait*"
	" allelic richness "		Allee
	"allel* frequenc*"		
	"number* of alleles"		
	"allel* number*"		
	beneficial + (allele* or mutation*)	genetic structure	
	resist* + (allele* or mutation*)		
	favourable + (allele* or mutation*)		
	favorable + (allele* or mutation*)		
	adapted + (allele* or mutation*)		
	adaptation + (allele* or mutation*)		
	"proportion* of *migrant*"		
	"hybrid* rate*"		
	"*migrant* proportion*"		
	"hybrid* proportion*"		
	"proportion* of hybrid*"		
	"inbreeding depression"	inbreeding depression	
	"drift load* "	drift load	
	"genetic load*"		

heterosis †	
s-allele*	
"self-incompatib*"	
overdominance	
heterozygo* advantage*	
distyl*	
tristyl*	
pin	
pins	
thrum*	
morph	
morphs	systems with balancing selection
heterostyl*	
gynodioec*	
MHC	
Major Histocompatibility Complex*	
"complementary sex determination"	
CSD	
genetic* compatib*	
genetic* incompatib*	
cytoplasmic male-sterility	
CMS	
balancing selection	
"negative frequency-dependent selection "	
"genetic* deterioration*"	
"genetic* swamping*"	
"genetic* takeover*"	
"genetic* aggression*"	migration load
"outbre* depression*"	
hybridization*	
"migrat* meltdown*"	
"migrat* load*"	

† heterosis may be due to drift load

CHAPITRE 3 : PROPORTIONS DE MALES DIPLOÏDES ET GENETIQUE DES POPULATIONS

Pour détecter un effet Allee démographique dû à la production de mâles diploïdes, nous allons étudier de très petites populations. Avant cela, nous avons cherché à savoir si un effet Allee élémentaire dû à la production de mâles diploïdes pouvait être présent dans des populations plus grandes et qui n'ont pas été créées dans le but de mettre en évidence un effet Allee. Pour cela, nous avons mesuré la diversité génétique et la proportion de mâles diploïdes dans des populations naturelles et captives. Auparavant, nous avions besoin d'un outil pour mesurer la ploïdie des mâles. C'est pourquoi nous avons développé des marqueurs microsatellites, qui permettent de mesurer à la fois la ploïdie des mâles et des paramètres de génétique des populations.

3.1 Méthodologie: développement de marqueurs microsatellites

19 marqueurs répartis en 2 PCR multiplexes ont été développés. Pour des individus issus de deux populations naturelles, tous les marqueurs ont été amplifiés et sont polymorphes. Ils comportent de 2 à 14 allèles, avec des fréquences d'allèles nuls inférieures à 10%. Aucun déséquilibre de liaison n'a été détecté et tous les loci sont à l'équilibre d'Hardy-Weinberg. Les marqueurs développés peuvent donc être utilisés pour effectuer des mesures classiques de génétique des populations.

Les marqueurs microsatellites peuvent être utilisés pour mesurer la ploïdie des mâles : si un mâle est homozygote à tous les loci, il est haploïde. Cependant, un mâle diploïde homozygote à tous les loci peut sera considéré à tort comme haploïde. Nous avons calculé la probabilité de faire cette erreur en utilisant une seule PCR multiplexe pour mesurer la ploïdie de mâles issus d'accouplements frère-sœur, qui ont donc une probabilité d'être homozygote plus élevée que des mâles moins consanguins. La probabilité de faire une erreur dans la mesure de la ploïdie est de 0,23%, ce qui est faible. Ce résultat a été confirmé en mesurant la ploïdie de 2 manières – génotypage et cytométrie en flux – chez des mâles issus d'accouplements frère-sœur. Pour les 39 mâles analysés, les deux méthodes donnaient le même résultat. La mesure de la ploïdie des mâles à l'aide de marqueurs microsatellites est donc considérée comme fiable.

3.2 Diversité génétique et proportion de mâles diploïdes en populations naturelles et captives

Afin de tester l'existence d'un effet Allee génétique du au système de détermination du sexe chez *V. canescens*, nous avons mesuré la richesse allélique aux marqueurs microsatellites et la proportion de mâles diploïdes dans plusieurs populations. La richesse allélique aux marqueurs microsatellites, mesurée avec une seule des PCR multiplexes, est considérée comme un proxy de diversité génétique au gène du CSD. Les mesures ont été effectuées dans des populations naturelles continentales (5 populations) et insulaires (1 population) et dans des populations captives (3 populations). On s'attend à une richesse allélique plus faible et une proportion de mâles diploïdes plus élevée dans les populations insulaires par rapport aux populations continentales, et dans les populations captives par rapport aux deux autres catégories de populations. En effet, la population insulaire est isolée des populations continentales, a probablement subi un goulot d'étranglement lors de sa fondation, et a une taille limitée par la taille de l'île. Les populations captives ont subi un goulot d'étranglement lors de la fondation. Des mesures de structure génétique entre populations ont aussi été effectuées pour mesurer le niveau d'isolement entre populations.

La population insulaire est fortement différenciée des populations continentales, même les plus proches de l'île. Toutes les populations continentales sont plus différenciées de la population insulaire que des autres populations continentales. Les populations captives sont fortement différenciées de leurs populations sources. Ces résultats confirment que la population insulaire est isolée des autres et que les populations captives ont subi de forts goulots d'étranglement à leur fondation. La richesse allélique est globalement plus élevée dans les populations continentales que dans les populations captives, avec une valeur intermédiaire pour la population insulaire. Cependant, dans toutes les comparaisons entre catégories de populations, certaines paires de population ne sont pas différenciées. Les proportions de mâles diploïdes varient entre 2% et 16%. Ces proportions sont similaires pour toutes les populations continentales. La population insulaire a une plus grande proportion de mâles diploïdes que les populations continentales. Dans les populations captives, la proportion de mâles diploïdes dépend de l'histoire des populations. Une population fondée peu avant l'analyse par plus de 100 femelles présente une très faible proportion de mâles diploïdes (2%). Dans les autres populations captives, les proportions de mâles diploïdes sont similaires à celle de la population insulaire. Une corrélation entre richesse allélique et proportion de mâles diploïdes a été observée.

Ces résultats suggère que les populations naturelles et captives de *V. canescens* peuvent être affectées par un effet Allee génétique du au système de détermination du sexe. Les deux conditions

d’un effet Allee génétique sont remplies. La diversité génétique est plus faible dans les population isolées et/ou goulotées, qui ont probablement une taille actuelle ou passée plus faible que les autres (condition 1). Il existe une relation négative entre diversité génétique et proportion de mâles diploïdes (condition 2). Quand la proportion de mâles diploïdes est élevée, le nombre moyen de descendants fertiles par femelle réduit par rapport à une population sans production de mâles diploïdes. Un effet Allee élémentaire est donc présent: dans les petites populations, les femelles produisent moins de descendants fertiles que dans les grandes populations.

Article II

Population genetics of a parasitoid wasp with single locus complementary sex determination: new microsatellite markers and genetic analyses

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Introduction

Genetic processes such as inbreeding depression or fixation of deleterious alleles lower the probability of persistence of small and/or isolated populations (Amos and Balmford 2001; Frankham 1998; Spielman *et al.* 2004). In some species of the order Hymenoptera, the sex determination system may cause a significant sensibility to losses of genetic diversity. All Hymenoptera are haplodiploid: females are diploid and develop from fertilized diploid eggs whereas males are haploid and develop from unfertilized haploid eggs. However, among the species where sexual differentiation depends on single-locus complementary sex determination (sl-CSD), only the diploids that are heterozygous at the sex-determination locus develop into females. Hemizygous haploids and homozygous diploids both develop into males (Whiting 1943). The problem arises from diploid males, which are generally unviable or sterile, and may therefore constitute a severe genetic load (Heimpel and de Boer 2008).

In populations with declining genetic diversity at the *csd* locus, the frequency of diploid males is expected to increase, to an extent where the consequent decrease in population growth, combined with demographic and environmental stochasticity, may drive small populations into an extinction vortex (Hein *et al.* 2009; Zayed and Packer 2005). However, the production of diploid males represents such a fitness cost that various individual behaviors have evolved to limit the production of diploid males (*e.g.*, natal dispersal and mate-choice; Ruf *et al.* 2011; Thiel *et al.* 2013; Whitehorn *et al.* 2009). Such behaviors may mitigate the diploid male vortex (Hein *et al.* 2009).

More than 50 studies have detected diploid males in natural populations (*e.g.* Liebert *et al.* 2005; Zayed *et al.* 2004). Male ploidy was often assessed with genetic markers. In addition to allow the assessment of male ploidy, genetic markers can serve to estimate three population parameters that underlie diploid male production: (1) within-population genetic diversity, (2) between-populations genetic structure and (3) mating structure. Small, bottlenecked or isolated populations are expected to have lower genetic diversity, and thus a higher proportion of diploid males. Three studies documented this trend, with lower allelic diversity and more diploid males in small insular populations of bumblebees (Darvill *et al.* 2012), in an introduced population of the invasive ant *Solenopsis invicta* (Ross *et al.* 1993), or in a captive population (sweat bee *Melipona scutellaris* Alves *et al.* 2011). Measures of between-population genetic structure provide information on the intensity of gene flow between populations. Well-connected populations are expected to produce less diploid males, as gene flow regularly brings new CSD alleles (Hein *et al.* 2009). Dispersal, the behavior that underpins gene flow, also limits inbreeding and the consequent diploid male production. Some Hymenoptera with sl-CSD have undifferentiated populations beyond a 100 km scale (Estoup *et al.* 1996; Zimmermann *et al.* 2011), suggesting good dispersal abilities. Finally, mating structures can be approached via measures of heterozygosity. A heterozygote excess,

compared to the genotypic frequencies under Hardy-Weinberg equilibrium, may indicate inbreeding avoidance.

Most evidence for diploid males in natural populations comes from social Hymenoptera. In these species, the dramatic bias in sex ratio toward females renders estimations of the proportion of diploid males very specific. In parasitoid wasps, the proportion of diploid males was assessed in only two related species: *Cotesia glomerata* (Ruf *et al.* 2013) and *C. rubecula* (de Boer *et al.* 2012). Given the key role that parasitoid wasps play by controlling the populations of herbivorous insects in natural ecosystems and agrosystems (Shaw and Hochberg 2001), and because parasitoid populations often experience small population size, either as a consequence of cyclic dynamics (Hassell 2000) or biological control introductions (Hopper and Roush 1993), the lack of field estimations on the occurrence of diploid males in parasitoids is surprising.

Venturia canescens (Hymenoptera: Ichneumonidae) is a parasitoid Hymenoptera with sl-CSD. This species is a model organism commonly used in laboratory studies on behavior, physiology and life-history traits (Desouhant *et al.* 2005; Harvey *et al.* 2001; Pelosse *et al.* 2007). In *V. canescens*, diploid males are fully viable and able to mate. However, diploid males are sterile, and females with whom they mate produce only sons, similarly to virgin females (A. Chuine, unpublished data). As a possible adaptation to limit the production of sterile males, female *V. canescens* discriminate against their brothers for mating (Metzger *et al.* 2010a). The presence of diploid males in natural or captive *V. canescens* populations has not been investigated yet, and little is known about the genetic structure of natural populations. So far, the sole population genetics study on *V. canescens* showed an absence of genetic structure at the geographic scale of the French Riviera (Mateo Leach *et al.* 2012; Schneider *et al.* 2002).

We therefore developed the present study with a threefold objective: (i) develop genetic markers in order to measure male ploidy and conduct population genetic studies on *V. canescens*; (ii) assess the genetic diversity and population structure of *V. canescens* populations under various conditions of population size; (iii) measure the proportion of diploid males in different populations. For this, we compared mainland, island and captive populations. We expect to observe lower genetic diversity and a higher proportion of diploid males in captive populations compared to insular populations, and in insular populations compared to mainland populations.

Materials and methods

Mainland populations

Male and female *V. canescens* were collected in seven mainland populations distributed in three sites in France and two in Spain (Table 1). The Pyrenees form a mountain range that separates French sites from Spanish sites, and the Alps separate the French site Valence from all other French sites (Fig 1). In summer 2010, *V. canescens* females were captured in Nice and Valence (France). Males were captured in late summer and autumn, in 2011 in Nice and in 2013 in Nice and Solliès (France), and Vinyols and Vila-Seca (Spain).

Insular populations

In late summer and autumn 2012, we sampled *V. canescens* males in six Mediterranean islands: Mallorca (Spain), Sicily (Italy), Malta, Gozo (Malta), Crete (Greece) and Cyprus. Several sites per island were searched. In late summer 2013, the same protocol was followed to capture males in Spanish and French islands (Table 1). No female traps were set up in island populations, although some females were captured with male traps.

Captive populations

To initiate captive populations (parasitoid mass rearing), females from two French populations (Valence and Nice) and a population from Israel were captured and maintained in the laboratory at the Institute Sophia Agrobiotech (Sophia-Antipolis, France) and the University of Claude Bernard Lyon 1 (France). We sampled males in each captive population: (i) a 25-30 generation old population founded with about 120 females from Nice, (ii) a 10-15 generation old population founded with about 100 females from Valence, and (iii) a 15-20 generation old population founded with 11 females from Israel (Table 1). Population maintenance was the same for all three populations: larvae of the host *Ephestia kuehniella* (Lepidoptera: Pyralidae) were reared in plastic boxes (8 × 12 × 25 cm) set with 250 g of wheat semolina and 50 mg of *E. kuehniella* eggs (about 2000 eggs) provided by Biotop (Livron-sur-Drôme, France). Each week, three boxes containing 2nd to 5th instar host larvae were inoculated with 50 male and 50 female *V. canescens* emerging from all available (about 6) rearing boxes.

Table 1. Studied populations: locality, type (mainland, island, captive population), geographic coordinates, host plant (Car, Fig, Wal, Pom, Che, Pea, Haz, Cit and Oli being respectively Carob, Fig, Walnut, Pomegranate, Cherry, Peach, Hazelnut, Citrus and Olive trees) and year of sampling (year of foundation between parentheses for captive populations), with the corresponding number of male or female individual sampled.

Populations						Sampling		
Country	**Name**	**Locality**	**Type**	**Geographic coordinates**	**Host plant**	**Date**	**Number of males**	**Number of females**
France	Val10	Valence	Mainland	N 44° 58' 21" E 4° 55' 39"	Che/Pea/ Haz	2010	0	31
	Nice10	Nice	Mainland	N 43° 41' 23" E 7° 18' 6"	Car	2010	0	44
	Nice11	Nice	Mainland	N 43° 41' 23" E 7° 18' 6"	Car	2012	190	0
	Nice13	Nice	Mainland	N 43° 41' 23" E 7° 18' 6"	Car	2013	90	21
	Sol13	Solliès	Mainland	N 43° 10’ 58" E 6° 2’ 55 "	Fig/Wal/ Pom/Che	2013	16	0
	CapNiceA	Nice	Captive	N 43° 41' 23" E 7° 18' 6"	Car	2013* (2011)	50	0
	CapNiceB	Nice	Captive	N 43° 41' 23" E 7° 18' 6"	Car	2013** (2011)	31	0
	CapVal	Valence	Captive	N 44° 58' 21" E 4° 55' 39"	Che/Pea/ Haz	2013 (2013)	50	0
Spain	VS13	Vila-Seca	Mainland	N 41° 7' 34" E 1° 8' 07"	Car	2013	58	2
	Vy13	Vinyols	Mainland	N 41° 6' 12" E 1° 2' 26"	Car/Oli	2013	33	2
	Mlc12	Mallorca	Island	N 39° 47' 58" E 2° 57' 53"	Car	2012	21	0
	Mlc13	Mallorca	Island	N 39° 47' 58" E 2° 57' 53"	Car	2013	38	3
Israel	CapIsr	Tel-Aviv	Captive	Not Available	Not Available	2013 (2011)	50	0
Greece	Cre12	Crete	Island	N 35° 11' 39" E 25° 2' 09"	Pom/Fig/ Cit/Oli	2012	0	1
	Cre13	Crete	Island	N 35° 11' 39" E 25° 2' 09"	Pom/Fig/ Cit/Oli	2013	0	1
Malta	Goz12	Gozo	Island	N 36° 3' 34" E 14° 16' 34"	Car	2012	0	2
Cyprus	Cyp12	Cyprus	Island	N 34° 39' 5" E 33° 0' 17"	Car	2012	0	1

* sampled in July 2013 ** sampled in October 2013

Figure 1: Location of studied natural populations.

Field traps

To attract parasitoid females, open cups containing a mixture of host-rearing medium (semolina) and larvae of the host *E. kuehniella* were hanged in trees. Infested semolina is impregnated with host kairomones and is very attractive for *V. canescens* females (Corbet 1971; Metzger *et al.* 2008). Females that visited the traps to oviposit were collected, killed and preserved in 96% ethanol.

V. canescens males are attracted by the synergy of pheromones by females and kairomones by hosts (Metzger *et al.* 2010b). Traps baited with extracts from these semiochemicals were designed to attract and capture males. A trap consisted of a 125 mm × 200 mm yellow sticky trap, in the center of which was hanged a vial containing the extract. To prepare the extract, 100 g of host-rearing medium containing larvae was soaked one hour in 200 mL of hexane. The solution was then filtered and one female was soaked in 100 µL for 3 hours. After removal of the female the extract was stocked at -20°C. A few hours before traps were exposed in the field, hexane was evaporated by heating the vial on a hot plate (This step was skipped for the 2013 campaign, after it was shown that evaporation occurred within a few hours in the field). As for females, traps were

hanged within host trees, and all *V. canescens* males that stuck on the traps were collected and preserved individually in 96% ethanol.

Except in Nice and Valence, which are known from several years to harbor *V. canescens* populations, we searched for trapping sites in other locations in the following way. We chose sites with at least 10 individual host plants (i.e. carob, fig, pomegranate, citrus, palm or walnut trees; Salt 1976). On each site, ten traps were evenly distributed. They were hanged in potential host trees at 50 to 150 cm from the ground. Traps were checked after 48h. In the case that one *V. canescens* (male or female) was found, 20 to 40 traps were added on the site.

Genetic markers

Development of a kit of markers

Using a DNEasy Tissue Kit from Qiagen (QIAGEN, Hilden, Germany), DNA was extracted from a single 1.5 mL tube containing 20 adult *V. canescens* recently collected from the Nice population and kept in the laboratory for a few generations. The obtained DNA solution was enriched in microsatellites and pyrosequenced by the company Genoscreen (Lille, France), following the protocol described in Malausa *et al.* (2011). Using the iQDD program (Meglecz *et al.* 2010), primer pairs were designed for 675 microsatellite loci (Malausa *et al.* 2011), among which 124 were screened in monoplex PCR on DNA of one thelytokous and six arrhenotokous *V. canescens* females from three populations: Valence (Table 1), Nice (Table 1) and Antibes (geographic coordinates: N 43° 33’ 51”, E 7° 7’ 28”). Six primer pairs designed by Mateo Leach *et al.* (2012) were also screened following the same protocol. The DNA extracts used for these monoplex PCR were all obtained using the DNeasy Tissue Kit of QIAGEN (Hilden, Germany). Each monoplex PCR contained 2µL of DNA, 5 µL of 2X QIAGEN Multiplex PCR Master Mix and 0.2 µM of each primer. The total volume was adjusted to 10 µL with ultrapure water. PCR was performed as follows: a step of denaturation at 95°C during 15 minutes, 25 cycles of 30s denaturation at 94°C, 90s annealing at 58°C and 60s extension at 72°C, and a final extension step of 30 minutes at 60°C. PCR products were separated on 2% agarose gel stained with ethidium bromide to detect amplification of a DNA fragment. For 37 successfully amplified loci, forward primers labeled with one of four different fluorochroms were ordered from Applied Biosystems (Carlsbad, USA) and used in a monoplex PCR with the DNA and conditions previously described. Two microliters of PCR product were added to 8.75 µL of Hi-Di formamide and 0.25 µL of GeneScan500 Liz size standard (Applied Biosystems). The mix was loaded on an ABI 3130XL genetic analyzer (Applied Biosystems) and alleles were scored using Gene Marker® version 1.75 (SoftGenetics, State College, USA). Monomorphic loci were discarded and the remaining loci were

combined according to their size and fluorochrom in several multiplex PCR that were tested on the same seven individuals and in same PCR conditions as previously described. Finally, we selected 19 microsatellite loci distributed in two multiplex PCR (Table 2).

Validation of markers for population genetics studies

To be used for population genetics studies, each marker must be polymorphic (minimum 2 alleles) with detectable alleles, and linked with no other marker or locus under selection. We therefore measured genetic polymorphism, frequency of null alleles, departure from Hardy-Weinberg equilibrium and linkage disequilibrium on females captured in 2010 in two mainland populations from Valence and Nice. Based on field observations, we assumed random mating in these populations (Driessen and Bernstein 1999; Schneider *et al.* 2003), and would inbreeding avoidance, as observed in laboratory conditions by Metzger *et al*. (2010a), occur in the wild, it should only impact Hardy-Weinberg equilibrium in small populations (Glémin *et al.* 2001).

DNA of each captured female was extracted with the PrepGem Insects kit (ZyGEM, Hamilton, New Zealand). PCR and genotype analyses were performed as previously described. For each population and microsatellite locus, the GENEPOP software version 4.2 (Rousset 2008) was used to measure the number of alleles, the expected and observed heterozygosity, and to test for Hardy-Weinberg (HW) equilibrium and linkage disequilibrium between loci. P-values for the latter tests were corrected by calculating the False Discovery Rate (FDR) on p-values grouped by marker for HW tests (19 groups of 2 p-values) and by applying Bonferroni corrections on p-values pooled altogether for the linkage disequilibrium tests (1 group of 38 p-values). We estimated null allele frequencies with the FreeNA program (Chapuis and Estoup 2007).

Validation of markers for measure of male ploidy

We used genetic markers from the multiplex PCR I (Table 2) to detect diploid males: if a male is found heterozygous for at least one locus, it is considered diploid (Armitage *et al.* 2010; Souza *et al.* 2010; Zhou *et al.* 2006). However, if a diploid male is homozygous for all loci, it will be misleadingly scored as haploid. To estimate the power of genetic markers to assess male ploidy, we calculated the probability that a diploid male is undetectable because it is homozygous at all 10 loci of multiplex PCR I. We also compared male ploidy assessed with genetic markers and with flow cytometry.

Table 2. Characteristics of two multiplex PCR amplifying 19 microsatellite loci in *Venturia canescens*.

			Primer sequences 5′−3′						
Multiplex	**Locus**	**Repeat motif**	**F-Primer**	**R-Primer**	**F 5′ label**	**Concentration (µM)**	**Size range (bp)**	**GenBank no.**	**Reference**
I	VC068	$(GA)_9$	TATCCTTCCAGCATTCGTCC	CTCGCTCGGTGGAACACTAC	FAM	0.1	104-120		This study
	Vcan071	$(CAA)_{11}$	CTCCTACGCACTCCCTTCAC	TTGTACGTTGGCACTTGAGC	FAM	0.1	223-254	GU053679	Mateo Leach, 2012
	VC092	$(AG)_9$	TGTTCGGCTCTTGCTGTAAGT	CTCTCGTCAATTGCGTCGT	FAM	0.1	284-307		This study
	VC094	$(GT)_8$	TCGATTGCTTGAATCCTCTG	CACATATTTTCCCTTGCACC	FAM	0.2	429-436		This study
	VC009	$(ACA)_{12}$	AACAGCAACAGCAACAGGTG	ACTTTTGCCACGTGATTTCC	VIC	0.1	313-337		This study
	VC036	$(TTC)_{12}$	GTCAGCGATACACGCACG	GTACGCCTCTTATTCTCGCG	NED	0.1	226-254		This study
	VC002	$(AG)_{11}$	TCCGTTTCGTCTCATTATAATTCA	ATGATTGCTCTGACCGCTTC	NED	0.4	324-342		This study
	VC001	$(AG)_{10}$	TTTCGCCAGTTTGCTGTAAG	AACGAAACGAAATTTACAATCG	NED	0.4	391-447		This study
	VC066	$(CAA)_7$	ACACATTTGAACTCGAATCGAA	TCCTCTTGAAGCTCAAATTGC	PET	0.2	87-90		This study
	VC060	$(CTT)_{12}$	TATCTCGCGTTCTATTCCGG	AGGCGCTGATTCGAAGTTAA	PET	0.2	206-231		This study
II	VC106	$(AG)_{11}$	CAAGCATGTATGTGATCGGTG	CGTAACTATTTCGCGTTGGC	FAM	0.2	89-97		This study
	Vcan73	$(TGT)_{15}$	GGTCCAACGGTACTTCCTGA	ACTTCCGTCAGCCCTACCTT	FAM	0.2	227-267	GU053681	Mateo Leach, 2012
	VC047	$(AG)_{11}$	ACCTGAGGGCACTATTCTGTTT	CGAAAGTTAATTTCTAGACCGAGC	VIC	0.2	141-157		This study
	VC031	$(TC)_{11}$	TCAGTCACTTAGTGCACTTGGAA	GGGTGGTGTAATAGAGCGAGG	VIC	0.2	227-261		This study
	VC006	$(AG)_8$	GACTAATGCAGGAGGTTGTCG	GGCACAGTTTATGTTTCAGCG	VIC	0.2	317-357		This study
	VC120	$CCCCT(CCCT)_2$ $(CT)_2CCCT(CT)_9$	CAATCGATCAACGATACATTCG	GCAGGGTAGCAGCTTAGTGG	NED	0.2	92-128		This study
	Vcan106	$(TC)_{24}$	CCTCATCTCGAGGGAGGATT	ATCGCGAGTTGCGTAGTTTC	NED	0.2	183-222	GU053714	Mateo Leach, 2012
	VC107	$(CAG)_5(CAA)_{12}$ $CAGAGG(TA)_2$	CAACATCACCAACAACACCA	CACTTGCACATGTCGTTGC	PET	0.2	85-108		This study
	Vcan088	$(CA)_{44}$	AGTAACCGGTCAGCCTTTGG	CACGTTCCAATTTCCACACA	PET	0.2	133-150	GU053696	Mateo Leach, 2012

We first calculated the probability that a diploid male produced by a brother-sister pair is homozygous for all 10 loci of multiplex I. This probability was computed from the allelic frequencies and inbreeding coefficient (Fis) of a sample of 30 females collected in a 3 to 5 generation-old captive population founded in autumn 2009 with about 40 females from Nice. These females constitute the F0 generation and had presumably mated at random in the population. Their offspring (generation F1) were constrained to mate between siblings. We computed the probability that an F2 male, produced via sibmating, is homozygous for all genetic markers from multiplex PCR I (see Annex 1).

We also compared ploidy measured on the same individuals by two methods: genotype analyses and flow cytometry. Measure of male ploidy by flow cytometry is based on DNA quantity in cell nuclei (haploid nuclei being expected to have twice less DNA than diploid nuclei) and is thus independent from genotypes at microsatellite markers. To increase the proportion of diploid males, brother-sister pairs were formed with individuals from a 15 to 20 generation-old captive population. Male offspring were collected, killed, and their body was cut: the thorax was used for genetic analyses and the head for flow cytometry. For genetic analyses, DNA was extracted; genotyping was performed with multiplex PCR I and genotypes were analyzed as previously described. Males with at least one heterozygous locus were considered diploid. Among these males, we selected 20 haploid and 19 diploid males and carried out flow cytometry analyses on them, as described in de Boer *et al.* (2007). Only the head of insects was used for flow cytometry, because endoreduplication doubles the quantity of DNA in cells of other parts of the body of haploid males, making haploid and diploid nuclei undistinguishable (Aron *et al.* 2005). To isolate cell nuclei, each insect head was crushed in 0.5 mL of Galbraith buffer by turning the B pestle 20 times in a Dounce tissue grinder maintained on ice. The homogenate was filtered through a 40µm cell strainer cap. Cell nuclei were stained with 20 µL propidium iodide at 1 mg/mL. Nuclei were analysed on a LSRII Fortessa flow cytometer (BD Biosciences, San Jose, USA) with an excitation wavelength of 561 nm. Using FACSDiva Version 6.1.3 (BD Biosciences), we analyzed 10 000 nuclei per sample in a region that excluded doublets and debris. Flow cytometric DNA-histograms of diploid females were used as reference to identify diploid males.

Population differentiation and allelic richness

For all individuals captured in mainland, island and captive populations, DNA was extracted, PCR performed with multiplex I and genotypes analyzed as previously described. To document genetic structure between populations, tests of population differentiation were performed. For mainland and island populations, we first tested differentiation at a local scale, and then

increased the distance between populations compared. We compared populations within site, and pooled them if they were not differentiated. The same procedure was applied within regions (France, Spain mainland and Spain Island), then within category (mainland, island) and finally between mainland and island. We expected differentiation to increase with distance in mainland. The island populations should be differentiated from the mainland ones, as the sea strongly limits gene flow. Captive populations (Valence and Nice) were compared to the mainland population they originate from. The differentiation between captive and source populations is a measure of the strength of the bottleneck populations underwent at foundation.

We used allelic richness as a measure of population genetic diversity because it can be standardized independently of sample size and calculated for a population of both haploid and diploid individuals. With FSTAT version 2.9.3.2, we computed a value of allelic richness per locus and per population. We used the pooled populations defined by population differentiation tests. Allelic richnesses were compared pairwise with exact Wilcoxon signed rank tests. Populations were compared within mainland populations, within captive populations and between the three categories. To detect a bottleneck, the Nice and Valence captive populations were also compared to their source populations.

Proportion of diploid males

The ploidy of each captured male was deduced from its genotype at microsatellite markers from multiplex PCR I. A male was considered diploid if it was heterozygous at one or more locus. The observed proportions of diploid males, for the different populations, were compared with pairwise Fisher exact tests. Pairs of populations displaying similar proportions of diploid males were pooled following the procedure described above. After pooling, the three categories of populations (mainland, island and captive) were compared. We tested the correlation between allelic richness and proportion of diploid males using a logistic regression.

Results

Insect sampling

Venturia canescens was found in all sites expect in the islands of Sicily, Malta, Porquerolles and Corsica (Table 1). Females were trapped on female traps in Nice and Valence. Males, and sometimes females, were found on male traps in Nice, Solliès, Vila-Seca, Vinyols and Mallorca. Males and females were used for measures of population differentiation and allelic richness. No male was sampled on male traps in Crete, Gozo and Cyprus. Only one or two females were found

on these sites (Table 1). Although they were genotyped, the small sample size did not allow population genetics analyses. These four females were homozygous for all 10 markers, except one female, captured in Crete in 2012, which was heterozygous for locus VC009. They may belong to the thelytokous subspecies of *V. canescens*, which is known to have a very low heterozygosity rate (Mateo Leach *et al.* 2012).

Validation of markers for population genetics studies

In females trapped in Nice (Nic10 population) and Valence (Val10 population) in 2010, all markers were polymorphic, with a null allele frequency lower than 10% for all loci and equal to 0 for half of loci in each population (Table 3). After false discovery rate correction, we found a significant departure from the Hardy-Weinberg equilibrium for three loci (VC009 for the Nic10 population, and VC060 and VC001 for the Val10 population) suggesting that they could be linked to a locus under selection. We nonetheless retained these loci because the HW equilibrium test was significant in one population only, so the departure from HW equilibrium may be due to the limited number of individuals sampled rather than microsatellite loci linked to a locus under selection. No linkage disequilibrium between loci was detected, suggesting that the microsatellite loci developed were independent from one another.

Validation of markers for ploidy measures

The probability that a diploid male produced by sibmating is homozygous for all microsatellite loci of multiplex I was low enough ($p = 0.0023$) to rely on microsatellites to assess male ploidy. This was confirmed by the congruence between flow cytometry and microsatellite genotyping. Haploid and diploid males were easily discriminated by flow cytometry, with diploid males presenting a similar profile to diploid females (Fig. 2). For the 39 males tested, the ploidy measured by flow cytometry matched that deduced from genotyping.

Table 3. Genetic diversity at 19 microsatellite loci for the Nice10 and Val10 populations. *n*: number of individuals analysed; H_E: expected heterozygosity; H_O: observed heterozygosity; HW P-value: P-value of the test for Hardy-Weinberg equilibrium; (*) P-value significant after FDR correction.

			Population							
			Nice10 (*n* = 44)				Val10 (*n* = 31)			
Multiplex	**Primer name**	**number of alleles**	**H_E**	**H_O**	**HW P-value**	**null allele frequency**	**H_E**	**H_O**	**HW P-value**	**null allele frequency**
I	VC068	6	0.750	0.727	0.738	0.012	0.585	0.591	0.306	0.000
	Vcan071	11	0.818	0.750	0.180	0.036	0.573	0.591	0.203	0.000
	VC092	7	0.644	0.705	0.085	0.000	0.417	0.477	0.975	0.000
	VC094	4	0.599	0.545	0.478	0.034	0.364	0.432	0.672	0.000
	VC009	7	0.705	0.864	0.010*	0.000	0.482	0.500	0.988	0.000
	VC036	7	0.547	0.568	0.492	0.000	0.311	0.273	0.238	0.027
	VC002	7	0.584	0.591	0.610	0.026	0.518	0.523	0.570	0.000
	VC001	11	0.607	0.500	0.084	0.080	0.469	0.364	0.009*	0.080
	VC066	2	0.493	0.386	0.219	0.069	0.349	0.318	0.721	0.024
	VC060	8	0.555	0.591	0.815	0.000	0.381	0.364	0.001*	0.018
II	VC106	4	0.719	0.750	0.901	0.000	0.522	0.477	0.315	0.015
	Vcan73	10	0.679	0.818	0.401	0.000	0.519	0.409	0.042	0.085
	VC047	5	0.580	0.636	0.699	0.000	0.431	0.364	0.158	0.027
	VC031	14	0.657	0.591	0.036	0.048	0.474	0.409	0.249	0.078
	VC006	11	0.680	0.773	0.622	0.000	0.417	0.409	0.750	0.000
	VC120	7	0.521	0.500	0.500	0.000	0.431	0.386	0.038	0.004
	Vcan106	14	0.885	0.886	0.142	0.008	0.601	0.659	0.939	0.000
	VC107	6	0.732	0.705	0.180	0.000	0.442	0.455	0.241	0.022
	Vcan088	8	0.652	0.682	0.572	0.009	0.364	0.386	0.715	0.000

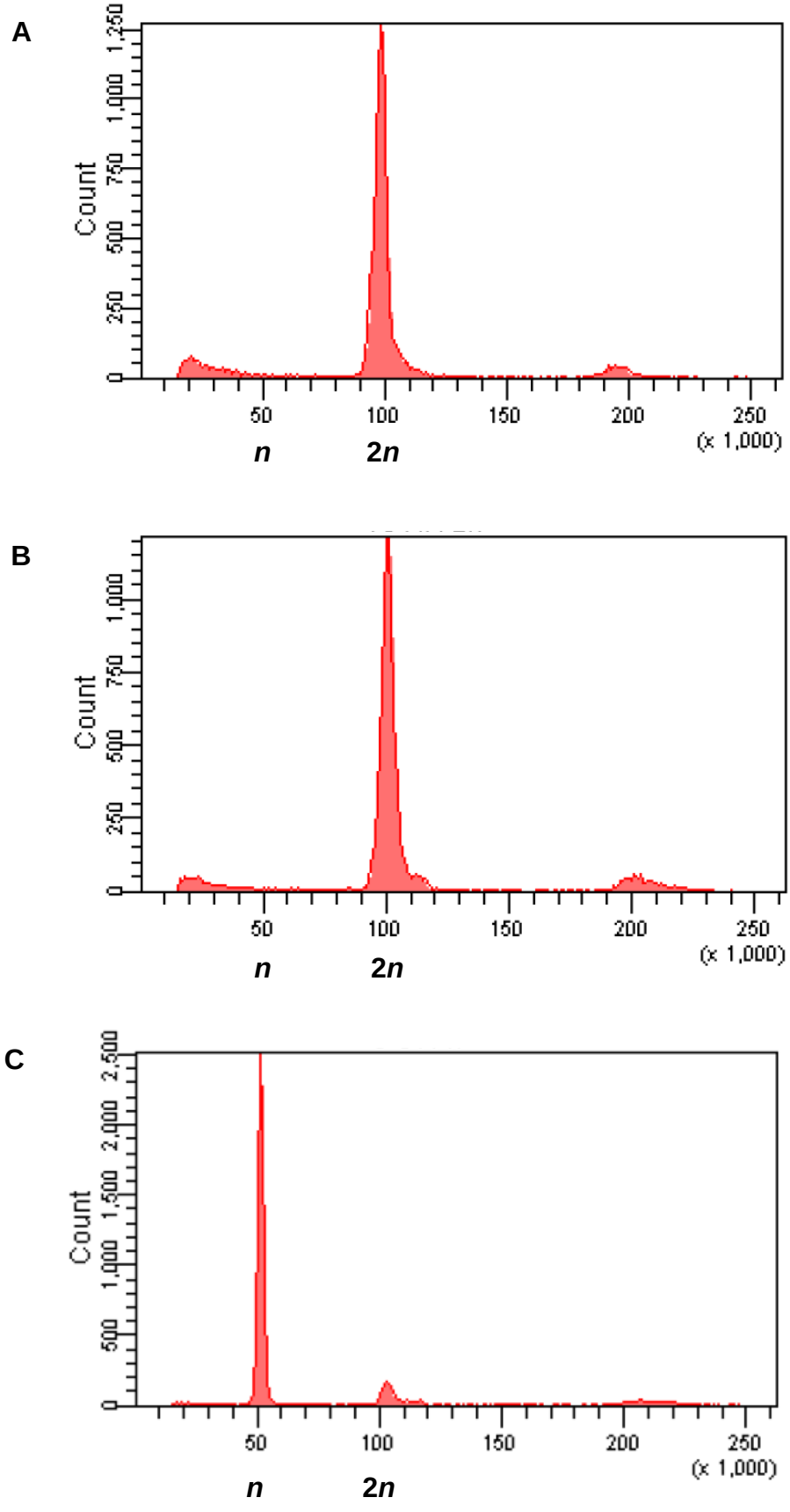

Figure 2. Flow cytometric histograms of the number of nuclei registered as a function of their fluorescence intensity (FI), for a representative female (A), diploid male (B) and haploid male (C). FI is expressed in an arbitrary unit calibrated to value 100 at the fluorescence intensity with the highest number of nuclei registered in females, which are known to be diploid.

Population differentiation

When they appeared statistically undistinguishable, we pooled populations from a same site, country or category (procedure detailed in table S1). After pooling, we obtain three mainland populations, each corresponding to an area of mainland France and Spain (Nice, Rhone Valley and mainland Spain) and one island population. As expected, the island population was strongly differentiated from all mainland populations, including populations from mainland Spain, which are the closest geographically (Table 4). Nice and Valence captive populations were all strongly differentiated from their source populations (Table 4). These captive populations probably underwent a strong bottleneck. Surprisingly, although they were sampled from the same captive population within a four-month interval, the two captive populations from Nice appeared differentiated (p= 0.005, Table S1).

Table 4: Pairwise tests of genetic differentiation (exact G-tests), after pooling undifferentiated populations (for details, cf. Table S1). Pooled populations are: Island (populations Mlc12 and Mlc13 from Mallorca), Nice (populations MB10, MB12 and MB13, from Nice), Rhone Valley (populations Sol13 and Val10 from the Rhône Valley, France) and Mainland Spain (populations VS13 and Vy13 from mainland Spain). Three comparisons are performed: between mainland populations, between island and mainland populations, and between captive populations and their source mainland populations.

Comparison			χ^2	p-value
Mainland populations				
SpMain	vs.	RhoneV	29.55	**0.042**
		Nice	37.46	**0.005**
Nice	vs.	RhoneV	37.46	**0.005**
Island vs Mainland populations				
Island	vs.	Nice	Inf.	< **0.001**
		RhoneV	Inf.	< **0.001**
		SpMain	Inf.	< **0.001**
Source vs. Captive populations				
Val10	vs.	CapVal	Inf.	< **0.001**
Nice	vs.	CapNiceA	Inf.	< **0.001**
		CapNiceB	61.59	< **0.001**

Allelic richness

We used allelic richness as a measure of genetic diversity (Table 5). Overall, mainland populations had the highest genetic diversity, and captive populations the lowest, with an intermediate diversity for island populations (Table 6). The Valence and Nice captive population had a lower allelic richness than their corresponding mainland population, suggesting a loss of alleles during the bottleneck of the foundation (Table 6). Among the captive populations, the population from Israel had a lower genetic diversity (2.3 alleles per locus) than all other populations (3.6 to 4.7 alleles per locus; Table 6), which can be explained by the low number of foundresses (11 females) for this population.

Table 5: Allelic richness per locus and mean allelic richness for various populations of *Venturia canescens*. Allelic richness was calculated based on a minimum sample size of 10 diploid individuals. The locus VC-036 was discarded because it was accidentally not amplified from individuals from the Mlc12 population.

	Locus									
Population	**VC068**	**Vcan71**	**VC092**	**VC094**	**VC009**	**VC002**	**VC001**	**VC066**	**VC060**	**Mean**
Mainland										
Nice	5.241	7.267	4.183	3.313	3.965	4.698	4.117	2.096	3.831	4.301
RhoneV	6.378	6.560	4.481	4.220	4.603	4.683	5.153	2.000	4.456	4.726
SpMain	5.560	6.449	4.362	2.863	4.433	4.989	4.500	2.000	4.514	4.408
Val10	6.201	6.542	4.778	3.567	4.708	4.747	5.032	2.000	4.549	4.680
Island										
Island	5.376	4.695	4.157	2.981	3.057	5.279	3.636	2.000	4.242	3.936
Captive										
CapIsr	1.826	2.822	2.727	1.985	2.727	2.890	1.840	1.000	3.303	2.347
CapNiceA	4.385	5.051	3.815	3.497	3.930	4.192	4.376	2.000	3.503	3.861
CapNiceB	4.534	6.428	3.333	2.769	3.000	3.970	2.000	2.000	4.689	3.636
CapVal	5.588	4.184	2.998	2.931	2.977	4.287	2.994	2.000	3.389	3.483

Table 6: Pairwise comparisons (exact Wilcoxon signed-rank tests) of allelic richness between the different populations sampled. When allelic richnesses are significantly different ($p < 0.05$), the population with the highest allelic richness is indicated in bold.

Comparison			p-value
Captive populations			
CapIsr	vs	**CapVal**	**0.004**
		CapNiceA	**0.004**
		CapNiceB	**0.004**
CapVal	vs	CapNiceA	0.195
		CapNiceB	0.844
CapNiceA	vs	CapNiceB	0.64
Island vs Mainland populations			
Island	vs.	Nice	0.426
		RhoneV	**0.039**
		SpMain	0.109
Island vs Captive populations			
Island	vs.	CapIsr	**0.004**
		CapVal	**0.039**
		CapNiceA	0.844
		CapNiceB	0.383

Comparison			p-value
Mainland vs Captive populations			
SpMain	vs.	CapIsr	**0.004**
		CapVal	**0.039**
		CapNiceA	0.055
		CapNiceB	**0.039**
Nice	vs.	CapIsr	**0.004**
		CapVal	**0.012**
		CapNiceA	0.074
		CapNiceB	0.074
RhoneV	vs.	CapIsr	**0.039**
		CapVal	**0.008**
		CapNiceA	**0.008**
		CapNiceB	**0.023**
Source vs. Captive populations			
Val10	vs.	CapVal	**0.008**
Nice	vs.	CapNiceA	0.074
		CapNiceB	0.074

Proportion of diploid males

Proportions of diploid males within the 11 sampled populations varied between 2% and 16% (Table 7). After pooling, we obtain one mainland population, one island population, and three captive populations (Table S2). As expected, the proportion of diploid males was higher in island populations than in mainland populations (Table 8). Captive populations varied in their frequency of diploid males. The captive population from Valence had a lower proportion of diploid males (2 %) than the population from Israel (16%, $p = 0.031$, Table S2). Only the captive population from Israel had a higher proportion of diploid males than the mainland population (Table 8). The captive population from Valence had fewer diploid males than the island population (Table 8). We found no increase in proportion of diploid males in the Nice captive population compared to its source mainland population (Table 8).

The proportion of diploid males was partially explained by genetic diversity. We found a significant negative correlation between allelic richness and the proportion of diploid male (LR χ^2 = 5.73, Df = 1, p = 0.017).

Table 7: Number of haploid and diploid males male and proportion of diploid males for each population

Population	Number of male		Proportion of male (%)
	Diploid	Haploid	Diploid
Mainland			
Nice11	12	178	6.3
Nice13	4	86	4.4
Sol13	1	15	6.3
VS13	4	54	6.9
Vy13	1	32	3.0
Island			
Mlc12	3	18	14.3
Mlc13	6	32	15.8
Captive			
CapNiceA	7	43	14
CapNiceB	2	29	6.8
CapVal	1	49	2
CapIsr	8	42	16

Discussion

In insects of the order Hymenoptera with single locus Complementary Sex Determination (sl-CSD), sterile diploid males are expected to become more abundant if the population genetic diversity of populations decreases. This process is at the core of the diploid male vortex, a positive feedback between demography and genetics that may sometimes drive small populations to extinction (Zayed and Packer 2005). The proportion of diploid males should be higher in small, isolated and/or bottlenecked populations, because small population size is generally associated with low genetic diversity. However, adaptive behaviors (dispersal, mate choice) should limit the production of diploid males, and hence, delay the occurrence of any consequent vortex (Hein *et al.* 2009).

Table 8: Pairwise comparisons (Fisher exact tests) of proportion of diploid males between the three categories of populations, after pooling of populations within each category. When differences are significant ($p < 0.05$), the population with the highest proportion of diploid males is in bold.

Comparison			p-value
Island vs. Mainland populations			
Island	vs.	Main	**0.013**
Island vs. Captive populations			
Island	vs.	CapNice	0.610
	vs.	CapVal	**0.020**
	vs.	CapIsr	1.000
Mainland vs. Captive populations			
Mainland	vs.	CapNice	0.085
	vs.	CapVal	0.497
	vs.	**CapIsr**	**0.013**
Source vs. Captive populations			
Nice	vs.	CapNice	0.132

Genetic markers allow the estimation of population isolation and genetic diversity. Genetic markers can also serve as a method to assess the ploidy of field-captured males, and hence, population-wide proportions of diploid males. We report here the development of new microsatellite markers for the parasitoid *Venturia canescens*. We used these markers to measure population differentiation, allelic richness and proportion of diploid males in mainland, island and captive populations, that is, three categories of populations for which genetic diversity and the proportion of diploid males is expected to vary. Overall, we found lower genetic diversity and higher proportion of diploid males in bottlenecked and isolated populations.

We developed 15 microsatellite markers for *V. canescens*, distributed in two multiplex PCR which also include 4 markers developed by Mateo Leach *et al.* (2012). These markers are suitable for population genetics study, and enable a precise measure of male ploidy. They add to the other microsatellite markers developed by Mateo Leach *et al.* (2012).

When comparing mainland and island populations, the island population of Majorca appeared genetically isolated from the mainland populations and had a slightly lower allelic

richness (one pairwise comparison significant out of three). The proportion of *V. canescens* diploid males was higher in Majorca Island than on the mainland. These results are congruent with the prediction that insular populations are isolated from the mainland populations. For this reason, as well as concurrent processes such as founder effects, smaller population size and stronger genetic drift, insular populations have lower genetic diversity and experience negative fitness consequences such as the production of diploid males.

For two geographic sites, we sampled both a captive population and the mainland population from which the former was derived. The two captive populations were genetically isolated from their source population. Globally, captive populations had a lower genetic diversity than island and mainland ones. Captive populations are thus isolated and genetically depauperate compared to wild populations. The proportion of diploid males varied dramatically among captive populations, which prevents us from drawing general conclusions from comparisons between captive and other population categories. The proportion of diploid males was higher in Israel (16%, 8/50) and Nice (11%, 9/81) populations than in the Valence one, where only 2% (1/50) of males were diploid. These differences can be partially explained by the history of captive populations. Populations from Nice and Valence were founded with a similar number of individuals, but the population from Nice was about twice older than the one from Valence. As a consequence from genetic drift, the population from Nice may thus have lost more CSD alleles. The population from Israel was founded with ten times fewer individuals than the two other populations, which explains its high proportion of diploid males.

Captive populations from Nice and Valence had lower genetic diversity than the Majorca Island population. However, the proportion of diploid males was lower in the captive population from Valence than on the island, suggesting that neutral genetic diversity, measured by microsatellite markers, is lost faster than diversity at the CSD locus. Ten to fifteen generations after foundation, the captive population from Valence had already lost neutral variability compared to its source population, but variability at CSD locus could have remained, as suggested by the low proportion of diploid males. The older population from Nice exhibits a tendency to lose both kinds of diversity.

CSD alleles are under negative frequency-dependent selection (Yokoyama and Nei 1979): rare alleles have a higher probability to be transmitted to the next generation. CSD alleles should therefore be less impacted by genetic drift (Glémin *et al.* 2005) than neutral markers. Nevertheless, we did find a negative relationship between allelic richness and proportion of diploid males, implying that, in our study populations, diversity at the CSD locus might be estimated by neutral genetic diversity.

Despite being more than 200 km apart, wild populations from Valence and Solliès were undifferentiated. This suggests good dispersal abilities of *Venturia canescens*. Surprisingly, population from Nice and Solliès, which are only 110 km apart, were differentiated. Maybe strong and frequent winds in the Rhône valley influence the connectivity between populations from Valence and Solliès.

We showed that, in the parasitoid wasp *Venturia canescens*, small, isolated or bottleneck populations have lower genetic diversity and higher proportions of diploid males. We therefore demonstrate for the first time in a parasitoid wasp, a relation between population size, genetic diversity and the proportion of diploid males. This relation is the core of Zayed & Packer's (2005) "diploid male vortex". Hence, if in turn, diploid male production affects population growth rate, small populations of *Venturia canescens* could be at high extinction risks. Could this explain the absence of *V. canescens* in small islands like Porqueroles, despite its presence on nearby mainland? The proportion of diploid males found in *V. canescens* is in the order of magnitude of estimations made in other species of parasitoids with sl-CSD: 10% in a native population of *Cotesia glomerata*, (Ruf *et al.* 2013) and 15% in an established biological control population of *C. rubecula* (de Boer *et al.* 2012). Evidently, a proportion of diploid males between 10 and 15 percent does not always affect the persistence of parasitoid populations.

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Annex: Calculation of the probability that an F2 male, produced by sibmating, is homozygous for all 10 markers from multiplex A.

We first calculated the probability that an F2 diploid individual is homozygous at one microsatellite locus, given the genotypes of its grand-parents (F0 generation) at this locus (Table A). Then, for each locus *k*, we calculated the probability that an F0 female is heterozygous at this locus as $P_k(Het_{F0}) = (1\text{-}Fis) \times He$ and the probability that she is homozygous as $P_k(Hom_{F0}) = 1 - P_k(Het_{F0})$, where He is expected heterozygosity at loci *k* under HW equilibrium. We calculated as follows the conditional probabilities that the F0 male carries at loci *k* a different (DA) or common (CA) allele with the F0 female, knowing that the female is heterozygous (Het_{F0}) or homozygous (Hom_{F0}):

$$P_k(CA \mid Het_{F0}) = \sum_{i=1}^{n} \sum_{\substack{j=2 \\ i<j}}^{n} \left(\frac{2 p_i p_j}{He} \right) (p_i + p_j)$$

$$P_k(DA \mid Het_{F0}) = 1 - P_k(CA \mid Het_{F0})$$

$$P_k(CA \mid Hom_{F0}) = \sum_{i=1}^{n} \left(\frac{p_i^2}{1 - He} \right) p_i$$

$$P_k(DA \mid Hom_{F0}) = 1 - P_k(CA \mid Hom_{F0})$$

p_i and p_j are the frequencies of alleles *i* and *j*, respectively. Using the proportions of F2 homozygous from table A, the probability that an F2 diploid is homozygous as locus *k* was: $P_k(Hom_{F2}) = P_k(Het_{F0})$ (0.5 P_k(CA| Het_{F0}) + 0.25 P_k(DA| Het_{F0})) + $P_k(Hom_{F0})$ (P_k(CA| Hom_{F0}) + 0.5 P_k(DA| Hom_{F0})).

The probability that a diploid F2 is homozygous at all 10 loci from multiplex A was:

$$P(Hom_{F2}) = \prod_{k=1}^{10} P_k(Hom_{F2}) .$$

Table A. Proportion of heterozygous diploid F2 at one microsatellite locus, according to the genotype of the F0 pair.

F0 female	Heterozygous AB								Homozygous AA	
F0 male	Different allele C				Common allele A				Different allele B	Common allele A
F0 genotypes	AB x C				AB x A				AA x B	AA x A
F1 genotypes	AC x A	AC x B	BC x A	BC x B	AA x A	AA x B	AB x A	AB x B	AB x A	AA x A
Diploid F2 genotypes	AA AC	AB BC	AB AC	BB BC	AA	AB	AA AB	AB BB	AA AB	AA
Proportion of homozygous diploid F2	0.25				0.5				0.5	1

Supporting information

Table S1: Pairwise tests of genetic differentiation (exact G tests) used to pool populations within a category. Alls tests have *Df* = 18 degrees of freedom.

Comparison	χ^2	p-value	Pooling of populations
Nice mainland populations			
Nice13 vs. Nice11	23.51	0.172	
Nice10	25.59	0.110	Nice (Nice)
Nice11 vs. Nice10	28.77	0.051	
Spain mainland populations			
VS13 vs. Vy13	15.60	0.620	Spain mainland (SpMain)
France mainland populations			
Val10 vs. Nice	63.19	< **0.001**	No pooling
Sol13	25.29	0.117	Rhone Valley (RhoneV)
Sol13 vs. Nice	51.82	< **0.001**	No pooling
RhoneV vs. Nice	61.06	< **0.001**	No pooling
Mainland populations			
SpMain vs. RhoneV	29.55	**0.042**	No pooling
Nice	37.46	**0.005**	No pooling
Island populations			
Mlc12 vs. Mlc13	13.02	0.790	Island (Island)
Nice captive populations			
CapNiceA vs. CapNiceB	37.5	**0.005**	No pooling

Table S2: Pairwise comparisons of proportions of diploid males (Fisher exact tests) used to pool populations with a category.

Comparison			p-value	Pooling of populations
Nice mainland populations				
Nice13	vs.	Nice11	0.783	Nice (Nice)
Spain mainland populations				
VS13	vs.	Vy13	0.650	Spain mainland (SpMain)
France mainland populations				
Sol13	vs.	Nice	1.000	France mainland (France)
Mainland populations				
France	vs.	SpMain	1.000	Mainland (Main)
Island populations				
Mlc12	vs.	Mlc13	0.650	Island (Island)
Nice captive populations				
CapNiceA	vs.	CapNiceB	0.471	Nice captive (CapNice)
Captive populations				
CapNice	vs.	CapIsr	0.434	No pooling
	vs.	CapVal	0.088	No pooling
CapVal	vs.	**CapIsr**	**0.031**	No pooling

CHAPITRE 4 : DEPRESSION DE CONSANGUINITE

La production de mâles diploïdes peut être considérée comme une forme de dépression de consanguinité car elle est moins fréquente lors d'accouplements entre non apparentés qu'entre apparentés, qui ont une plus forte probabilité de porter le même allèle au gène du CSD. Il se peut aussi que *V. canescens* soit affecté par d'autres formes de dépression de consanguinité qui concernent uniquement les femelles car les mâles sont haploïdes et ne peuvent donc pas être consanguins. Les mâles diploïdes peuvent toutefois être considérés comme des « femelles ratées » souvent consanguines. Dans ce chapitre, nous étudions la dépression de consanguinité chez les femelles et les mâles diploïdes de *V. canescens*. C'est un préalable nécessaire à l'expérience de test du « diploid male vortex » qui permettra la recherche d'effets Allee génétiques et démographiques dans des populations expérimentales de *V. canescens*. La dépression de consanguinité autre que la production de mâles diploïdes peut réduire le taux d'accroissement des populations, tout comme la production de mâles diploïdes. Si les femelles sont affectées par la dépression de consanguinité, il faudra en tenir compte lors de l'interprétation des résultats de l'expérience de test du « diploid male vortex ». Il est également important de mesurer la fitness des mâles diploïdes pour savoir si l'un des scénarios testés par les modèles théoriques (Zayed & Packer, 2005, Hein *et al.*, 2009) peut être appliqué à *V. canescens*. Ce sera le cas si les mâles diploïdes, dont on sait déjà qu'ils sont entièrement viables (Beukeboom, 2001), sont stériles, ont la même probabilité d'accouplement que les mâles haploïdes et engendrent des descendants triploïdes non viables. Il se peut au contraire que les mâles diploïdes ne s'accouplent pas ou moins souvent que les mâles haploïdes et/ou qu'ils soient partiellement fertiles. Dans ce cas, il serait utile d'intégrer les informations collectées dans un modèle théorique pour obtenir des prédictions pour les résultats de l'expérience de test du « diploid male vortex ».

4.1. Dépression de consanguinité chez les femelles

Nous avons recherché l'existence de dépression de consanguinité chez les femelles de *V. canescens*. On s'attend à ce que la dépression de consanguinité soit faible chez un haplo-diploïde car les allèles délétères peuvent être purgés lors de la phase haploïde de production des mâles (Henter 2003). Toutefois, cette purge n'est pas efficace pour les traits spécifiquement femelles. La dépression de consanguinité, bien qu'en moyenne moins élevée que chez les diploïdes, a d'ailleurs été détectée chez des haplo-diploïdes, sur des traits spécifiques ou non aux femelles (Henter, 2003).

De plus, la production de mâles diploïdes est une forme importante de dépression de consanguinité chez *V. canescens*.

Par croisements contrôlés, nous avons obtenu des femelles avec trois niveaux de consanguinités : descendantes de parents frères et sœurs, de parents collectés au hasard dans une même pop ou de parents collectés au hasard dans deux populations différentes. Plusieurs composantes de la fitness ont été mesurées sur les femelles et leurs parents : probabilité d'accouplement des parents en 45 minutes d'observation, production de filles, sex ratio de la descendance, taille du corps, symétrie, charge en œufs à l'émergence et à la mort, longévité.

La charge en œufs à l'émergence, un trait spécifiquement femelle, était affectée à la fois par la dépression hybride et la dépression de consanguinité, avec une différence de 20% entre femelles issues de croisements frère-sœur et de croisements aléatoires intra-population. Cependant, la charge en œufs à la mort ne présentait pas de différences entre types de croisements. Nous voyons deux explications possibles pour ce résultat. Soit la maturation des œufs au cours de la vie des femelles a comblé la différence entre types de croisements, soit des œufs surnuméraires sont régulièrement éjectés par les femelles, même en l'absence d'hôtes. Cela a déjà observé chez la forme asexuée de *V. canescens* et pourrait masquer une différence de charge en œufs entre types de croisements. La proportion de mâles dans la population augmentait avec le niveau d'apparentement des parents, ce qui confirme que la production de mâles diploïdes est une forme de dépression de consanguinité chez *V. canescens*. Les autres composantes de la fitness mesurées n'étaient pas affectées par la dépression de consanguinité.

Quelles sont les conséquences de ces résultats pour l'expérience de test du « diploid male vortex » ? Les femelles issues de croisements frère-sœur ont 20% d'œufs matures en moins à l'émergence comparées aux femelles issues de croisements aléatoires intra-population. Même si la différence entre les deux types de croisements persiste au cours de la vie des femelles, les femelles consanguines ont tout de même une charge d'une centaine d'œufs à leur mort. Etant donnés les taux de survie entre œuf et adulte chez *V. canescens* (Metzger, 2008), une femelle consanguine peut produire environ 75 descendants adultes par femelle, ce qui fait un taux d'accroissement toujours très élevé. Il est ainsi possible que les femelles soient limitées en hôtes avant d'être limitées en œufs et que la dépression de consanguinité sur la charge en œufs n'ait pas d'impact sur le taux d'accroissement des populations. Nous nous attendons donc à un effet négligeable de la dépression de consanguinité autre que la production de mâles diploïdes dans les populations expérimentales fondées pour tester le « diploid male vortex ».

Article III

Inbreeding depression in a parasitoid wasp with complementary sex determination

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Abstract

Inbreeding and inbreeding depression are key processes in small or isolated populations and are therefore central concerns for the management of threatened or (re)introduced organisms. Haplodiploid species of the order Hymenoptera have a particular status with regard to inbreeding depression. Although recessive deleterious alleles that are expressed in males should be purged, an alternative form of inbreeding depression exists in species with single-locus complementary sex determination (sl-CSD). Under sl-CSD, genetically-related parents have a high probability of producing sterile sons instead of healthy daughters. In this article, we study inbreeding depression in *Venturia canescens* (Hymenoptera: Ichneumonidae), a parasitoid wasp with sl-CSD. We used a crossing design to manipulate relatedness according to three levels: within-family, between-family and between-population. For each level, several fitness components were measured on parents and female offspring. We found a 20% level of inbreeding depression on potential fecundity at emergence. Inbred crosses also yield a higher proportion of males, as expected in a species with sl-CSD. Mating probability, presence of daughters among offspring, body size, symmetry and longevity were unaffected by inbreeding.

Introduction

Inbreeding depression is an adverse consequence of inbreeding, *i.e.*, the reproduction of genetically related individuals, which refers to the lower fitness of inbred compared to outbred individuals [1,2]. Systematic inbreeding occurs routinely in some mating systems, but it should then combine with adaptations alleviating inbreeding depression [3,4]. In contrast, when mating is random, inbreeding can have dramatic effects. In that case, inbreeding increases mechanically with decreasing population size [5], and when it results in inbreeding depression, inbreeding becomes a major component of extinction vortices that threaten small populations [6-8]. Inbreeding depression is therefore a process that links population genetics and population dynamics [9,10] and should, for this reason, be a central concern in population management [11-14].

In this paper, we study inbreeding depression in a parasitoid wasp. Planned introductions of parasitoids into novel environments for the biological control of insect pests cause abrupt bottlenecks that impede population establishment [13]. In addition, parasitoids have cycling dynamics resulting from tight demographic feedbacks with their hosts [15] and/or dramatic seasonal variations of environmental factors that yield recurring small population sizes. For these reasons, the study of inbreeding depression in parasitoid wasps is a relevant endeavor from both academic and applied perspectives.

Although superdominance – *i.e.* the higher fitness of heterozygous individuals – and favourable epistasis – *i.e.* a fitness advantage resulting from the interaction among different loci – [16-18] cannot be excluded, inbreeding depression is mainly due to the expression of recessive deleterious alleles that are brought to a homozygous state by inbreeding [1,19]. The fixation of such deleterious alleles is commonly detected by comparing the fitness of offspring originating from parents with various degrees of genetic relatedness. Relatedness between parents can be assessed via their pedigree, but access to such information in natural populations is difficult. A widespread alternative is therefore to compare the fitness of offspring from closely related parents (inbred crosses, obtained via selfing or sib mating) to that from parents randomly chosen from either the same population [ex: 20,21] or from different populations [9]. Theory predicts that inbreeding should affect life-history traits more than morphological traits [22], and some data are consistent with this prediction [but see 22,23,24].

Parasitoid wasps belong to the Hymenoptera order and are therefore haplodiploid, which raises two interesting twists in the study of inbreeding depression. First, in common with other haplodiploid organisms, parasitoid wasps are expected to be partially immune to inbreeding depression. The reason is that recessive deleterious alleles are exposed to selection at each generation *via* haploid males, so that haplodiploids should suffer a much more benign genetic load

than diploids [25-27]. Although the purge of deleterious alleles makes sense and is generally supported by data [28-31], it does not totally impede inbreeding depression [see for instance 32]. One reason is that the purge should concern neither female-specific genes or traits [33] nor loci with superdominance [34].

The second twist in the study of inbreeding depression in parasitoid wasps comes from the sex determination system of some species of the order Hymenoptera. This system relies on the complementarity of alleles at a single locus and is therefore sensitive to inbreeding. With single-locus complementary sex determination (sl-CSD), individuals that are diploid and heterozygous at the CSD gene develop into females, whereas individuals that are either diploid and homozygote or haploids (and hemizygotes) develop into males. Haploid males are normal but diploid males are generally unviable or sterile [35,36], so that the complementary sex-determiner gene (*csd*) displays an extreme form of superdominance. The production of diploid males being more likely from related parents, sl-CSD is often referred to as a form of inbreeding depression, potentially elevating the base extinction risk in haplodiploids by over an order of magnitude higher than that caused by inbreeding depression in threatened diploids [37]. But what complicates the picture is that sl-CSD favors the evolution of inbreeding avoidance [38-40], which in turn increases heterozygosity and may therefore slow down the purging of recessive deleterious alleles involved in female traits.

The solitary parasitoid wasp *Venturia canescens* (Gravenhorst) has a single-locus complementary sex determination system with viable but sterile diploid males [41]. In this species, females partially avoid their brothers for mating [38]. In the field, hosts of *V. canescens* are scarce and dispersed, and parasitism rates are low, so adult parasitoids emerge solitarily; females then search for hosts and males search for females [38,42]. This suggests a panmictic mating system due to rare encounters between siblings (despite sib-mating avoidance behaviors). Accordingly, genetic analyses with microsatellite markers show no departure from Hardy-Weinberg equilibrium in the studied populations (C. Vayssade, unpublished data). These characteristics, combined with a good knowledge of the species biology [43,44] make *V. canescens* an appropriate biological model to study inbreeding depression in a species with sl-CSD.

Our objectives are to study inbreeding depression in *V. canescens*, through the production of diploid males and variations in several other fitness traits. To detect inbreeding depression, we performed three types of crosses displaying three levels of relatedness. For each cross, we measured mating probability and offspring sex ratio, as well as several life-history and morphological traits on female offspring, more susceptible to inbreeding depression than haploid males. We expect to observe inbreeding depression shown by a negative relationship between the relatedness of parents and the value of fitness components.

Materials and methods

Venturia canescens strains and rearing

Venturia canescens Gravenhorst (Hymenoptera) is a solitary endoparasitoid of several Lepidoptera species living in dried fruits such as figs, carobs and dates [45]. In the wild, two subspecies of *V. canescens* occur in sympatry. One is thelytokous and produces only females; the other is arrhenotokous and produces both males and females [46]. In the latter, the proportion of males is about 40%. Males can mate several times whereas females are monandrous [38]. In summer 2010, *V. canescens* females from the arrhenotokous strain were collected at two locations separated by 230 km and a major mountain range (the Alps). About 150 females were captured in Gotheron, near Valence, France (N 44° 58' 21", E 4° 55' 39") and 120 females were caught at Mont Boron, near Nice, France (N 43° 41' 23", E 7° 18' 6"). No permission is required to collect samples of this species. These females were used to found two laboratory populations, further referred to as "Valence" and "Nice". Parasitoids were reared in plastic cages (8×12×25 cm) containing 2^{nd} to 5^{th} instar larvae of the host *Ephestia kuehniella* (Zeller) feeding on organic wheat semolina. Honey and water were applied on the cage net to feed adult wasps. Rearing and experiments were carried out at a temperature of 24±1°C under a LD 16:8 photoperiod. To limit sib-mating and genetic erosion during rearing (*i.e.*, about 4 generations), parasitoids were distributed across several cages and each new generation was initiated with a mix of adults emerging from all the different cages.

Crossing method

To measure the intensity of inbreeding depression on *V. canescens*, we performed crosses with three degrees of relatedness (Fig. 1). At generation G_0, families were formed by randomly pairing males and females within each population. Families were distributed across four blocks, corresponding to different weeks. Within a block, all pairs were formed the same day. We formed a total of 140 pairs, distributed in 10 pairs per population for blocks 1 to 3, and 40 pairs per population for block 4. At generation G_1, for each of the 140 families, three virgin females were paired, each with a different male: (1) a brother (within-family cross); (2) an unrelated male from the same population (between-family cross); (3) a male from the other population (between-population cross). Morphological and life-history traits were measured on their female offspring (generation G_2).

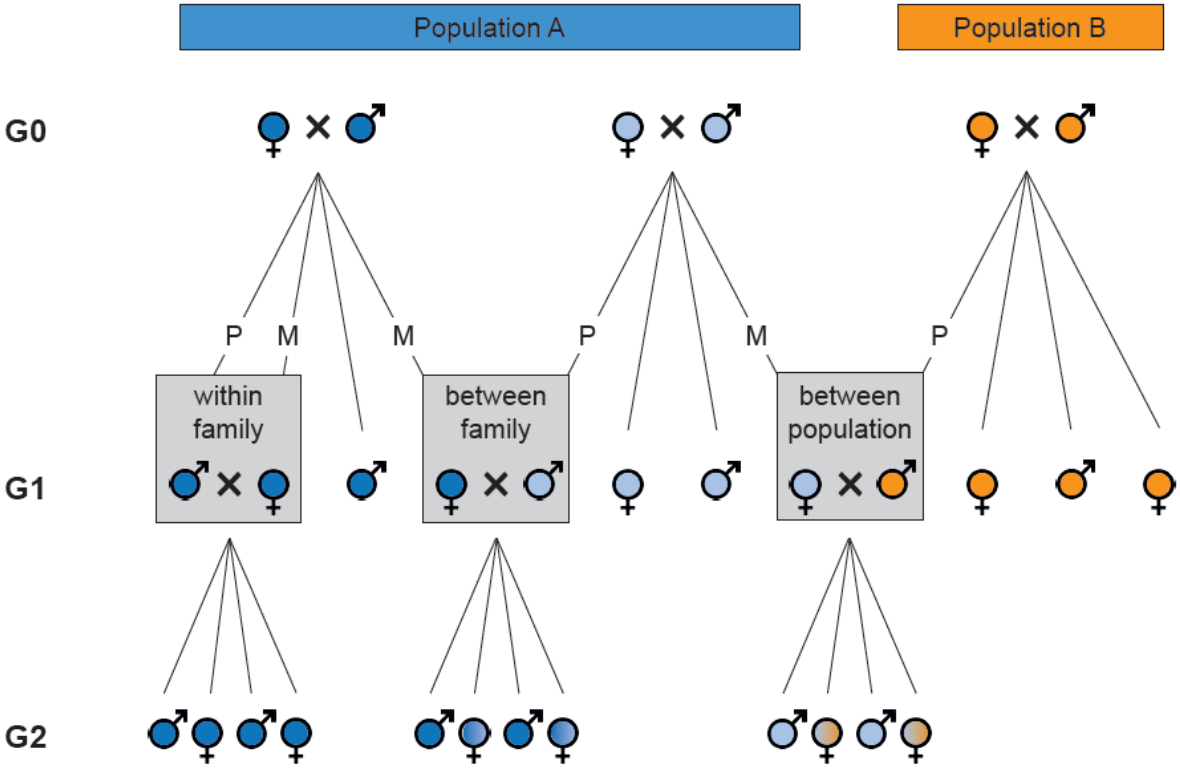

Figure 1. Cross design. At generation G_1, three types of cross were realized, each based on a different degree of relatedness between the parents. Mating probability and offspring sex ratio were measured on G_1 individuals. Morphological and life-history traits were measured on individuals of the G_2 generation. M indicates the maternal family and population, and P the paternal family and population.

For both G_0 and G_1 crosses, parents were collected within 30 minutes following emergence. As mating is scarce during this period, the individuals collected were presumably virgin. For mating and egg-laying, each G_0 pair was isolated for two days in an individual cage (10×7×2.5 cm) with many hosts. For G_1 crosses, all females were paired 1-4 days after emergence [i.e., before they become reluctant; Metzger47] (*i.e.*, before they become reluctant; Metzger, 2008). Moreover, the mating protocol for G1 crosses was adapted to allow estimation of mating success: each female was placed in a tube containing hosts and semolina with three males from the same family, a situation promoting mating in *V. canescens*. Individuals were observed 45 minutes or until mating had occurred (*i.e.* when the female remained still and did not reject the male during mounting). The female and the male she mated with (or a randomly selected male if mating had not been observed) were then placed in a vial without hosts in order to increase the probability that females were inseminated. After three days, the female was enclosed four hours in a cage containing 100 host larvae at the 3rd or 4th stage to produce the G_2 generation. Males and females were provided with food and water during all the experiment.

Variables measured

To assess the effect of inbreeding on individual fitness, eight variables were measured on either G_1 parents or G_2 offspring. On G_1 (parents), two indices of mating success were assessed: (1) the proportion of females that mated during the 45 minutes of direct observation and (2) the proportion of females that produced daughters. On G_2 (offspring), sex ratio was measured to detect the production of diploid males, which is expected from brother-sister mating. Moreover, two G_2 females were randomly selected in each family. One was frozen within 15 min from emergence and served to measure potential fecundity at emergence. The other was kept alive and used to measure potential fecundity at death and longevity. To estimate potential fecundity, ovaries were dissected under a binocular microscope and mature eggs were counted. Eggs were considered mature if they were in the oviducts, and immature if observed in the ovarioles or calyx. As there is no egg resorption in *V. canescens* [48], potential fecundity at death represents the number of eggs matured along the female's life. To measure longevity, females were placed in a 10×5 cm tube with water but no food and no host. Survival was checked every two hours between 9:00 am and 5:00 pm. The time of death was set as 1:00 am for females that died overnight. Size and symmetry were measured at emergence and at death. For this, we measured the length in µm of left (L) and right (R) hind tibias under a binocular microscope (×4) with the software AxioVision version 4.8 (Carl Zeiss). The asymmetry *A* of the hind tibias was calculated as $A = 2\times|L-R|/(L+R)$.

Statistical analyses

Eight response variables were analyzed, either on parents: mating probability, presence of daughters and proportion of males among offspring, or on daughters: body size, symmetry, potential fecundity at emergence, potential fecundity at death and longevity. The five explanatory variables were: type of cross, population - arbitrarily defined as the mother's population for between-population crosses (M; Fig. 1) - maternal family (M; Fig. 1), paternal family (P; Fig. 1) and block. To detect inbreeding depression, the effect of parental relatedness (type of cross) on each response variable was measured by fitting a generalized linear mixed-effects model to the data. Block, maternal family and paternal family were considered as random effects. Maternal and paternal families were nested within block and this structure was accounted for in the models. Type of cross, population, and interaction between cross and population were analyzed as fixed effects. Body size as a main effect or in interaction with cross and population was also included as a fixed effect in analyses of potential fecundity and longevity [in V. canescens as in most parasitoids, fecundity and longevity are positively correlated with body size; 44].

A model with a binomial distribution of errors and a logit link function was used for mating, presence of daughters, and sex ratio. Size and fecundity at death were analyzed with a normal distribution and an identity link function, and fecundity at emergence with a Poisson distribution and a log link function. Because generalized linear mixed models cannot be implemented with Gamma distributions, log-transformed values of symmetry and longevity were analyzed with a normal distribution and an identity link function. Two data points were excluded from the analyses: a point with extremely high value for potential fecundity at emergence and another with extremely low value for body size in the longevity data set.

For each variable, we first selected the most parsimonious model with regard to random effects. In a second step, we tested the hypotheses of null value of coefficient for each fixed effect. The random effects to be removed from the model were selected by likelihood ratio tests, with the fixed part of the model containing all fixed effects. If all random effects were removed, a standard generalized linear model was fitted. Type III Wald χ^2 tests were calculated on the model containing the selected random effects and all fixed effects. When tests revealed a significant effect for cross type, least square means (LSM) were estimated for each cross type, in order document inbreeding depression. Least-squares means were compared pairwise by Z tests and the p-values were adjusted using the Tukey method. All analyses were conducted with the lme4, lmerTest and lsmeans packages in the R statistical software [49]. All values in the text are given as mean ± standard error of the mean (SEM).

Results

The type of cross influenced neither the parent's probability of mating in 45 minutes (0.51 ± 0.04, Table 1A), nor the presence of daughters among their progeny (0.68 ± 0.03, Table 1B), but it did influence the offspring sex ratio (Table 1C, Fig. 2). Within-family crosses yielded a higher proportion of males (LSM = 0.67) than between-family (LSM = 0.58) and between-population crosses (LSM = 0.46). Between-family crosses also produced a higher proportion of males than between-population crosses.

Table 1. Generalized linear models and generalized linear mixed model for (A) mating probability, (B) presence of daughters among progeny, and (C) offspring sex ratio.

Model	Df	χ^2	Pr(> χ^2)
A. Mating probability (binomial errors, N = 176)			
Cross	2	0.29	0.8669
Maternal population	1	0.61	0.4330
Cross × maternal population	2	3.02	0.2206
B. Presence of daughters (binomial errors, N = 251)			
Cross	2	1.85	0.3962
Maternal population	1	1.78	0.1820
Cross × maternal population	2	2.71	0.2584
C. Sex ratio (binomial errors, M VC = 0.74, P VC = 0.86, N = 171, n_M = 75, n_P = 108)			
Cross	2	10.74	0.0046
Maternal population	1	0.37	0.5440
Cross × maternal population	2	0.71	0.6997

Details are provided in parentheses for each response variable: error distribution, variance components (VC) for the random effects selected (M = maternal family; P = paternal family), number of observations (N) and number of levels for random effects (n_M= number of maternal families; n_P = number of paternal families).

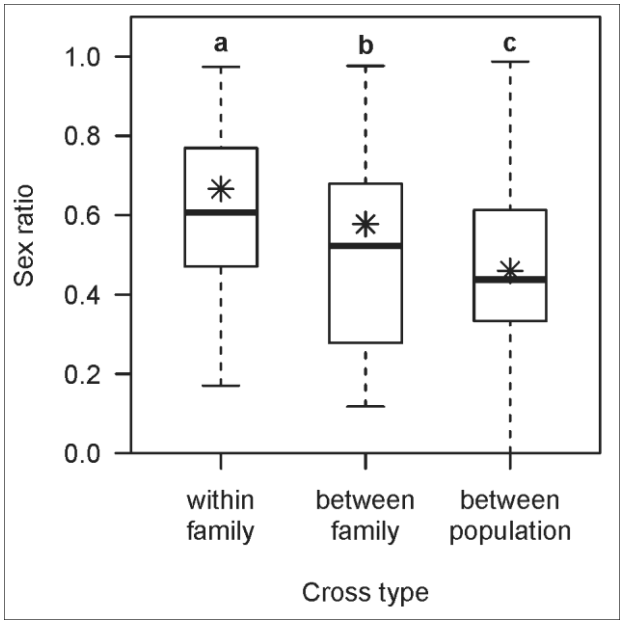

Figure 2. Boxplot of sex ratios for the three cross types. The bottom and top limits of the box are the 0.25 and 0.75 quartiles, respectively, and the bold black line indicates the median. Whiskers represent the minimum and maximum values. Asterisks show the model predictions for the type of cross with maternal and paternal families as random effects. Different letters indicate a significant difference of least-square means (within-family / between-family: $z = -2.91$, $P = 0.0102$; within-family / between-population: $z = -6.47$, $P < 0.0001$; between-family / between-population: $z = 4.03$, $P = 0.0002$).

The type of cross did not significantly influence body size (1907 ± 7.7 µm, Table 2A) and symmetry of offspring (0.95 ± 0.09 µm, Table 2B), nor did it affect their longevity (109 ± 3.5 h, Table 3C). The only strong effect of cross type on offspring fitness was found on potential fecundity at emergence (Table 3A, Fig. 3). Overall, females from between-family crosses had 20% more eggs in their ovarioles (LSM = 29.4) than females from within-family (LSM = 23.8) and between-population (LSM = 23.3) crosses. This effect of cross type was absent for potential fecundity at death, although a similar trend was present, with a higher mean potential fecundity for between-family crosses. Potential fecundity at death displayed higher values and within-cross type variance than potential fecundity at emergence (Fig. 3).

Quite surprisingly, the correlation between potential fecundity at emergence and body size varied according to the type of cross. Potential fecundity at emergence increased with body size for within-family and between-family crosses but not for between-population crosses (Fig. 4A). In contrast, potential fecundity at death increased with body size for all cross types (Fig. 4B).

Table 2. Generalized linear models for (A) body size and (B) symmetry.

Model	Df	F	Pr(> F)
A. Size (normal errors, N = 128)			
Cross	2	1.68	0.1915
Maternal population	1	0.91	0.3418
Cross × maternal population	2	2.18	0.1175
B. log(symmetry) (normal errors, N = 128)			
Cross	2	0.34	0.7097
Maternal population	1	0.26	0.6107
Cross × maternal population	2	0.40	0.6679

For each response variable, details are given in parentheses: error distribution and the number of observations (N).

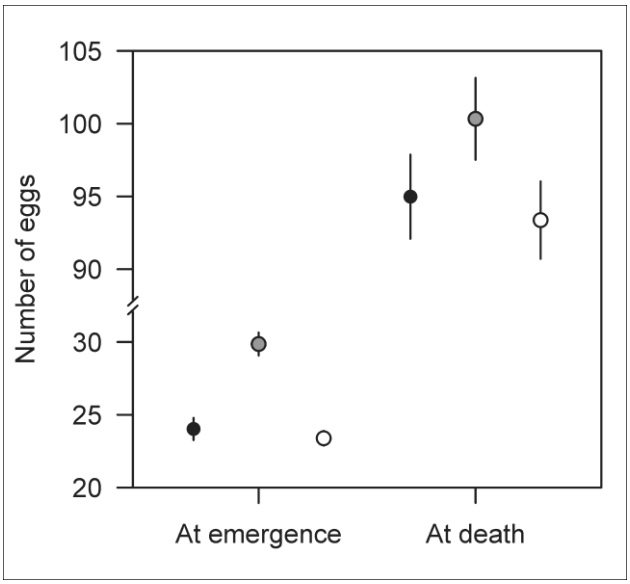

Figure 3. Means ± SEM of potential fecundity at emergence and at death. Means and SEM are calculated for observed values minus the predictions for random effects of a model with cross type, body size and their interaction as fixed effect and paternal and maternal populations as random effects (at emergence) or a model with body size as fixed effect and maternal population and block as random effects (at death). Means for within-family, between-family and between-population crosses are represented by black, grey and empty circles, respectively. For potential fecundity at emergence, least-square means were higher for between-family crosses than for other crosses (within-family / between-family: z = 2.92, P = 0.0097; within-family / between-population: z = -0.26, P = 0.9627; between-family / between-population: z = 3.14, P = 0.0049).

Table 3. Generalized linear mixed models for (A) potential fecundity at emergence, (B) potential fecundity at death and (C) longevity.

Model	Df	χ^2	Pr(> χ^2)
A. Potential fecundity at emergence (Poisson errors, M VC = 0.07, P VC = 0.10, N = 118, n_M = 58, n_P = 85)			
Cross	2	9.40	0.0091
Maternal population	1	0.90	0.3418
Body size	1	1.45	0.2284
Cross × maternal population	2	5.17	0.0756
Cross × body size	2	9.45	0.0089
Maternal population × body size	1	0.87	0.3518
B. Potential fecundity at death (normal errors, M VC = 191.5, B VC = 252.5, N = 113, n_M = 58, n_B = 4)			
Cross	2	0.11	0.9480
Maternal population	1	0.28	0.5979
Body size	1	6.22	0.0126
Cross × maternal population	2	0.04	0.9800
Cross × body size	2	0.08	0.9599
Maternal population × body size	1	0.41	0.5226
C. Log(longevity) (normal errors, B VC = 0.05, N = 115, n_B = 4)			
Cross	2	4.62	0.0993
Maternal population	1	0.32	0.5696
Body size	1	3.97	0.0464
Cross × maternal population	2	4.33	0.1150
Cross × body size	2	4.82	0.0898
Maternal population × body size	1	0.29	0.5892

For each response variable, details are given in parentheses: error distribution, variance components (VC) for the random effects selected (M = maternal family; P = paternal family; B = block), number of observations (N) and number of levels for random effects (n_M = number of maternal families; n_P = number of paternal families; n_B = number of blocks).

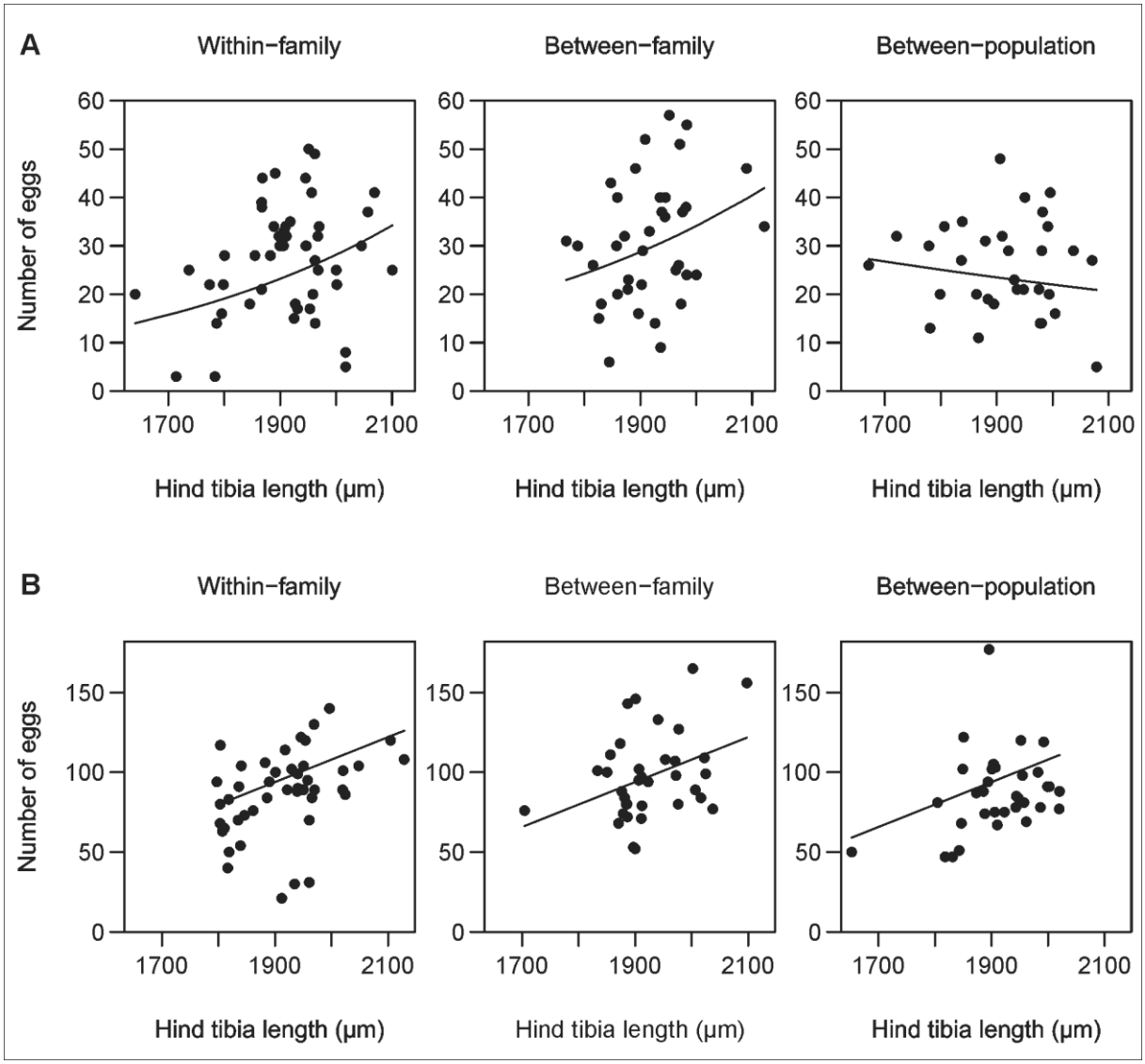

Figure 4. Potential fecundity (A) at emergence and (B) at death as a function of body size for the three cross types. Circles represent observed values and lines represent the predictions of the fixed part of the model with cross and body size as fixed effects and maternal and paternal families as random effects.

Discussion

Some organisms are more sensitive to inbreeding than others [9,50]. A common assumption is that haplodiploidy alleviates the consequences of inbreeding depression because the expression and subsequent counter-selection of deleterious recessive alleles in haploid males significantly reduces the genetic load. Data tend to support this general expectation: haplodiploid insects and mites do suffer less from inbreeding depression than diploid insects [28]. It is therefore no surprise that archetypal cases of systematic inbreeding are documented in haplodiploids such as pollinating fig wasps and parasitoids from the order Hymenoptera [51-53].

Careful analyses of inbreeding depression may nevertheless reveal subtle but interesting departures from this widespread view. First, although haplodiploids should be less prone to inbreeding depression, they may not be totally immune to adverse consequences of inbreeding, and both case studies and meta-analyses provide congruent evidence for non-negligible levels of inbreeding depression in haplodiploids [25,27,28,54]. Second, although the purging of load via haploid males may be efficient for genes underpinning many phenotypic traits, it may have no influence on genes that are expressed specifically in females [33]. And beyond haplodiploidy, inbreeding depression is also expected to be higher in life-history traits than in morphological traits [22,23]. Third, the beneficial effect of the haploid phase of haplodiploids is not expected to impact on alternative mechanisms of inbreeding depression such as superdominance or epistasis. A good example is the single-locus complementary sex determination (sl-CSD) of many hymenopteran insects, which produces a form of inbreeding depression caused by the very low fitness of individuals that are homozygous at the sex determination locus, although no one allele at that locus is in itself deleterious. Such alternative mechanisms can have dramatic consequences: the extinction risk resulting from sl-CSD in haplodiploids is predicted to be an order of magnitude higher than that produced by inbreeding depression in threatened diploids [37]. This adds a fourth twist to the analysis of inbreeding depression in hymenopteran haplodiploids: alternative mechanisms such as sl-CSD promote the evolution of sib mating avoidance [38,39], which should in turn result in higher heterozygosity at the population level, and potentially, a slower purge of deleterious alleles. Altogether, these different processes tone down the pervasive assumption that inbreeding depression is a minor problem for haplodiploid organisms.

From experimental manipulations of inbreeding coefficients among parents, we reveal here a small but significant level of inbreeding depression in the parasitoid wasp *Venturia canescens*. Females from sib mating emerged with 20% fewer eggs than females descending from unrelated parents of the same population. Interestingly, between-population crosses yielded a lower potential fecundity than between-family crosses which suggest that both inbreeding depression and outbreeding depression can be at play in *V. canescens*. This pattern fits Bateson's theory [55] of an optimal genetic distance between parents that maximizes the fitness of offspring. Such reasoning should however be taken with caution given the relatively low genetic and phenotypic distance between the two populations considered. Using 10 microsatellite markers, we indeed found a small but significant Fst of 0.013 between *V. canescens* populations from Nice and Valence (Vayssade, unpublished data). In other parasitoid species, inbreeding depression on fecundity was found in two different species of *Trichogramma* [25,32], *Uscana fumipennis* [28] and *Nasonia vitripennis* [56].

Despite the strong reduction of fecundity at emergence for inbred females, we observed no effect of inbreeding on fecundity at death. Because egg resorption does not occur in *V. canescens*

[48], two hypotheses can explain this observation. The first is that host-deprived females eject supernumerary eggs, as shown for the thelytokous subspecies of *V. canescens* [57]. Outbred females could have ejected more eggs than inbred ones, which could have leveled-down differences caused by inbreeding depression on potential fecundity at death. The second hypothesis is that inbred females have a slower egg maturation rate and complete their stock during their adult life. Such a situation would be particularly interesting insofar as egg maturation can be traded off with other activities such as flight, which depend on the same energy reserves [58]. Under such a hypothesis, inbred females would have reduced dispersal abilities. One way to untangle these alternative hypotheses is to measure realized fecundity, as it was done in other studies showing inbreeding depression for fecundity in parasitoids [25,28,32].

For offspring of within-family and between-family crosses, we observed a positive correlation between body size and fecundity at emergence. Such a relation is well-documented [59]. Here, however, this correlation was absent for between-population crosses. We have no explanation for this and will only mention that Henter [28] found a somewhat similar result with *U. fumipennis:* a positive relationship between body size and fecundity for females issued of brother-sister crosses but not for females from between-family crosses. Such results underline the necessity to investigate the possible effects of inbreeding depression not only on single traits, but also on correlations among traits.

Our study adds novel evidence for sl-CSD in *Venturia canescens* [41]. The proportion of males among offspring increased with increasing genetic relatedness among parents. On average, sibmating yielded an offspring sex ratio 63% of males which is very close to the 65% expected if we assume sl-CSD and 40% unfertilized eggs, as found in *V. canescens* [47]. Although we did not measure the ploidy of male offspring, this change in offspring sex ratio is likely due to higher proportion of diploid males as a consequence of increased homozygosity. In *Venturia canescens*, diploid males are similar to haploid males in most respects, but are completely sterile (A. Chuine, C. Vayssade, A. Auguste, E. Desouhant and X. Fauvergue, unpublished data). Hence, the increased proportion of diploid males among offspring with increased genetic relatedness among parents also fits the definition of inbreeding depression.

This study is the first report of inbreeding depression other than the production of diploid males in a parasitoid species with sl-CSD. We detected inbreeding depression on potential fecundity at emergence. Our study also confirmed that sl-CSD is a strong form of inbreeding depression in *V. canescens*. Consequently, in addition to producing less female offspring because of sl-CSD, inbred crosses produce female offspring with a reduced potential fecundity at emergence, although maybe compensated across adult life. *Venturia canescens* thus seems strongly affected by inbreeding depression.

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4.2. Dépression de consanguinité chez les mâles diploïdes

L'objectif de ce travail est de mesurer la fitness des mâles diploïdes et de détecter d'éventuelles adaptations réduisant l'impact de la production de mâles diploïdes sur la fitness, comme l'évitement par les femelles des accouplements avec un mâle diploïde ou la fertilité partielle des mâles diploïdes. Nous avons ensuite examiné les conséquences pour la dynamique des populations de la fitness des mâles diploïdes et des éventuelles adaptations détectées.

Des croisements frère-sœur ont été réalisés pour obtenir des mâles diploïdes et haploïdes. Plusieurs composantes de la fitness ont été mesurées sur ces mâles puis leur ploïdie a été déterminée par génotypage. Les composantes de la fitness mesurées sont la taille du corps, la symétrie, la survie juvénile, la longévité adulte et différentes traits comportementaux mesurés lors de tests de détection de femelles en tunnel et vol, de tests d'accouplement en situation de non choix et de tests d'accouplement en situation de choix dans des cages comportant 20 mâles, certains haploïdes et d'autres diploïdes. La probabilité d'accouplement après 24h en présence d'une femelle vierge, le transfert de sperme et le nombre de descendants femelles ont également été mesurés.

Les mâles diploïdes sont similaires aux mâles haploïdes pour la plupart des composantes de la fitness mesurées sauf trois. Le temps de latence avant accouplement est quatre fois plus long pour les mâles diploïdes que pour les haploïdes. La production de descendants des mâles diploïdes est anecdotique (une femelle triploïde a été engendrée) et donc considérée comme nulle. Le succès d'accouplement des mâles diploïdes en situation de compétition est deux fois plus faible que celui des mâles haploïdes. Les femelles accouplées à un mâle diploïde produisent autant de descendants que les femelles accouplées à un haploïde, à la différence que tous les descendants sont des mâles, comme si la femelle était vierge.

Les mâles diploïdes représentent un coût pour les femelles qui les produisent car ce sont des descendants stériles produits à la place des descendants femelles, mais aussi, dans certaines conditions, pour les femelles avec qui ils s'accouplent. Dans une grande population panmictique avec sex ratio équilibré, comme c'est le cas dans certaines populations naturelles de *V. canescens* (Chapitre 3), les mâles et les femelles ont la même probabilité d'accouplement. Il n'y a donc pas de coût à produire uniquement des mâles. Par contre, dans les petites populations, les variations stochastiques du sex ratio favorisent les femelles qui produisent des descendants des deux sexes (Taylor & Sauer 1980; Verner 1965), car l'un ou l'autre sexe peut se retrouver en majorité et voir sa fitness moyenne réduite par la compétition pour l'accès à l'accouplement. Chez *V. canescens*, dont les femelles sont monoandres mais les mâles polygynes, ceci est vrai surtout quand les mâles sont majoritaires, ce qui arrive plus souvent avec la production de mâles diploïdes. L'expérience de test

du « diploid male vortex » sera réalisée avec des petites populations. On s'attend donc à ce que la production de mâles diploïdes ait un coût à la fois pour les mères des mâles diploïdes et pour les femelles accouplées avec eux. On observerait alors deux effets Allee génétiques : quand la taille de population diminue, les femelles produisent moins de descendants fertiles (mères des mâles diploïdes) et produisent des descendants dont la fitness est plus faible (mères pseudo-vierges).

Dans les modèles de dynamique des populations avec CSD (Hein *et al.* 2009 ; Stouthamer *et al.* 1992; Zayed & Packer, 2005), aucun scénario n'envisage que les mâles diploïdes aient une probabilité d'accouplement réduite par rapport aux mâles haploïdes, ni que les femelles accouplées aux mâles diploïdes soient pseudo-vierges. Les conséquences de cette situation pour la dynamique des populations ont été analysées à l'aide d'un modèle individu-centré similaire à celui élaboré par Zayed & Packer (2005). Nous avons créé un nouveau scénario dans lequel les femelles accouplées à des mâles diploïdes sont pseudo-vierges. Un paramètre « probabilité d'accouplement des mâles diploïdes » a aussi été créé et l'impact de ce paramètre sur la probabilité d'extinction des populations a été mesuré grâce à une analyse de sensibilité. Cette analyse montre que la probabilité d'extinction des populations augmente avec la probabilité d'accouplement des mâles diploïdes. Dans de très petites populations avec un nombre de descendants par femelle élevé, la probabilité d'extinction est plus élevée quand les femelles accouplées avec un mâle diploïde sont pseudo-vierges que quand elles produisent des descendants triploïdes non viables. Pour d'autres valeurs de taille de population et de taux d'accroissement, les deux scénarios ont des probabilités d'extinction similaires

Pour tester le « diploid male vortex », nous utiliserons des populations à capacité de charge faible où le nombre moyen de descendants par femelle peut être élevé à cause d'une faible mortalité. Il s'agit donc d'un scénario favorisant l'apparition du « diploid male vortex », qui est un effet Allee démographique fort.

Article IV

Sterile males in a parasitoid wasp with complementary sex determination: from fitness costs to population extinction

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Introduction

Small populations are the centers of a number of intrinsic processes that make them extinction-prone, so that one direction in Conservation Biology, referred to as the small population paradigm, aims at understanding the nature and consequences of these processes (Caughley, 1994). Some are of strict demographic nature (demographic stochasticity, environmental stochasticity) while others are genetic (drift, inbreeding, selection). Behavioral processes are also implied in the fate of small populations. Some behaviors are disrupted at low population size and yield a decrease in population growth rate; these behaviors often underpin Allee effects (Berec *et al.*, 2007; Fauvergue, 2013; Stephens & Sutherland, 1999); other behaviors including mate-choice, multiple-mating and dispersal, interact with inbreeding and in that respect, participate to the genetics of small populations (Pusey & Wolf, 1996; Quader, 2005; Tregenza & Wedell, 2002). Heated debates have arisen on whether or not small populations are driven to extinction by stochastic events and demographic processes before genetic factors impact them (Caro & Laurenson, 1994; Lande, 1988; Spielman *et al.*, 2004). Extinctions may nevertheless rarely be the consequence of any single mechanism, but are rather triggered by feedbacks between multiple processes that interact to produce extinction vortices (Berec *et al.*, 2007; Fauvergue *et al.*, 2012; Gilpin & Soulé, 1986; Hedrick *et al.*, 1996). In the present article, we investigate on behavioral responses to inbreeding depression in a parasitoid wasp, and the consequences these responses may have on the extinction of small populations.

Inbreeding is a process that perfectly illustrates feedbacks between demography, genetics, and behavior. When populations decline, the number of different families becomes smaller and, under random mating, inbreeding, that is, the reproduction among genetically related individuals, fatally increases (Malécot, 1969; Willi & Fischer, 2005; Wright, 1931). Inbreeding yields decreased heterozygosity, and, as a potential adverse consequence, inbreeding depression (Crnokrak & Roff, 1999; Grueber *et al.*, 2008; Hansson & Westerberg, 2002; Hedrick & Kalinowski, 2000; Reed & Frankham, 2003). Inbreeding depression affects life-history traits that are related to demographic parameters (Keller & Waller, 2002) and may in turn combine with other processes such as demographic stochasticity to drive small populations into an extinction vortex (Bijlsma *et al.*, 2000; Brook *et al.*, 2002; Frankham, 1995a; b; Frankham & Ralls, 1998; Keller & Waller, 2002; Saccheri *et al.*, 1998). Adapted behaviors such as mate-choice and dispersal may nonetheless alleviate the adverse genetic consequences of reduced population size (Pusey & Wolf, 1996; Quader, 2005; Tregenza & Wedell, 2000) and hence, defuse the threat of extinction vortices.

The sex determination system of some species of the order Hymenoptera appears as an interesting paradigm to investigate on feedbacks between demography, genetics and behavior. In many species, single locus complementary sex determination (sl-CSD) is at the origin of a severe

form of inbreeding depression. Under sl-CSD, individuals that are diploid and heterozygous at the complementary sex-determiner gene (*csd*) develop into females, whereas individuals that are either diploid and homozygote or haploids and hemizygotes develop into males. Haploid males are normal, but diploid males are typically unviable or sterile (Cook, 1993; Heimpel & de Boer, 2008; van Wilgenburg *et al.*, 2006) (but for exceptions seeCowan & Stahlhut, 2004; Elias *et al.*, 2009). The expected proportion of unfit diploid males being higher in the offspring of genetically related parents (for instance, 12.5% among the progeny of full sibs), sl-CSD is a particular form of inbreeding depression based on superdominance: no single allele at the *csd* gene is intrinsically deleterious, but the lower fitness of homozygous individuals compared to heterozygous nonetheless generates a severe genetic load (Charlesworth & Willis, 2009). Sl-CSD is at the origin of the "diploid male vortex" whereby a decrease in population size results in a decrease in heterozygosity at the *csd* gene, an increase in the proportion of diploid males, a decrease in population growth rate, and if severe enough, a further decrease in population size (Zayed & Packer, 2005). The diploid male vortex elevates the expected extinction risk in haplodiploids by over an order of magnitude higher than that caused by inbreeding depression in threatened diploids (Hedrick *et al.*, 2006; Zayed & Packer, 2005). Hence, complementary sex determination tone down the pervasive belief that haplodiploid organisms are immune to inbreeding depression.

Zayed & Packer (2005) predicted the probability of extinction under single-locus complementary sex-determination to be sensitive to the life history and behavior of diploid males. If diploid males are unviable, their production is analogous to a decrease of female immature survival (because diploid males abnormally develop from fertilized eggs that should normally develop into females). If diploid males are viable but sterile, their production may have a two-fold effect spanning over two generations: first, they induce a decrease in the production of daughter for parents that shared an allele at the *csd* gene, and second, they induce a decrease in the production of daughters for females with whom they mate (assuming that eggs fertilized by diploid males are triploid and therefore, unviable). The set of parameters used by Zayed & Packer (2005) yielded an increase in mean extinction rate from 2.3%, resulting from stochastic events only, to 69% in the case of unviable diploid males, and up to 171% in the case of viable but sterile males. In addition, simulation models show that adapted mate-choice, e.g., discrimination against diploid males, significantly reduces extinction risk (Hein *et al.*, 2009). Hence, for species with sl-CSD, the fate of small populations depend on the survival and reproductive behavior of diploid males as well as the ability of females to discriminate diploid from haploid males.

We report here a series of experiments aimed at assessing the fitness of diploid males in a parasitoid wasp with sl-CSD, *Venturia canescens*. We show that in *V. canescens*, diploid males are similar to haploid males on most morphological, life-history and behavioral traits considered.

Nonetheless, diploid males take longer to mate in individual tests, and congruently, have a lower mating success when competing with haploid males. We also discovered a scenario different from the ones primarily hypothesized by Zayed & Packer (2005): in *V. canescens*, females inseminated by diploid males do not produce fewer offspring, but produce only sons. At the population level, we show with a simulation model that population extinction is strongly impacted by the mating success of diploid males. Hence, our study illustrates how the genetics of small populations can lead to individuals with different behaviors, and how these behaviors then impact on population dynamics, with consequences as dramatic as population extinction.

Materials and Methods

Biological system

Venturia canescens Gravenhorst (Hymenoptera: Ichneumonidae) is a parasitoid of pyralid moths including *Ephestia kuehniella*, *Plodia interpunctella* and *Ectomyelois ceratoniae* (Salt, 1976). Immature stages of the parasitoid develop from moth larvae inside desiccated fruits such as carobs, dates, medlars and figs, as well as husks from nuts and almonds (Salt, 1976). Adults can live several weeks during which males search for females and females search for hosts (Desouhant *et al.*, 2003; Metzger *et al.*, 2010b). The mating system of *V. canescens* has many attributes of panmixis. Because both the number of pyralid larvae per fruit and the proportion of parasitized hosts are low (Driessen & Bernstein, 1999), males and females generally emerge from distant sites and must search for one another actively. Field and laboratory experiments have shown that males use volatiles from hosts and females, synergistically, to orient toward the latter, whereas females orient toward volatiles emitted by their hosts (Metzger *et al.*, 2010b). Although sibmating is partially avoided (Metzger *et al.*, 2010a), within-population genetic structures, inferred from the frequency of 10 microsatellite loci in two different populations, suggest no departure from Hardy-Weinberg equilibrium (C. Vayssade, unpublished data). Altogether, these data reflect a mating system where males search actively for females and use oviposition sites as a point of rendezvous.

Although mated females are as attractive to males as are virgin females (Metzger *et al.*, 2010b), females mate only once in laboratory conditions. Consequently, mate choice is expected to be a major component of female fitness. Casual observations show that upon encounter, males perform a stereotypical courtship behavior (van Santen & Schneider, 2002) in the course of which females can eject them with their hind legs and escape from mounting (Metzger, 2008). A clear demonstration of female mate-choice in *Venturia canescens* is the reluctance of females to mate with their brothers, or with genetically unrelated individuals but in the presence of volatiles from their brothers (Metzger *et al.*, 2010a).

Individual *V. canescens* used in this study came from a mass-rearing initiated 9-10 generations before experiments with about 100 females captured near Nice, on the French Riviera (43°41'18"N, 7°18'10"E, altitude 130 m). Wasps were reared on second to fifth instar *Ephestia kuehniella* larvae that were themselves reared from eggs provided by commercial breeders (Biotop, Livron-sur-Drôme, France). Hosts were fed with organic wheat semolina and parasitoids with honey and water. Both were maintained in plastic boxes (300 × 100 × 100 mm) at 25±1 °C and 45±5 % RH, with a 16:8 L:D photoperiod. All males tested had been isolated in a glass vial 24 h after emergence and had been fed with honey and water.

General methods

In order to contrast haploid and diploid males, we used sibmating crosses to increase the number of diploid males available. Under sl-CSD and sibmating, half of the parents share a common allele at the *csd* locus (matched mating) and subsequently, half of their fertilized eggs develop into diploid males (Cook, 1993). Overall and assuming that females fertilize half of their eggs, brother-sister mating is expected to yield 50% haploid males, 12.5% diploid males and 37.5% diploid females (or, when assessed only among males, 20% should be diploids). Families were initiated with randomly chosen males and virgin females freshly emerged from the mass rearing (F0 generation). Each pair was enclosed 24h with hosts in a petri dish. Among offspring (the F1 generation), brothers and sisters were similarly paired and enclosed with hosts for oviposition. Male offspring of the F2 generation were tested in different experiments, and then genotyped to infer their ploidy.

Altogether, for the following experiments, we produced about 750 F2 males from about 150 F1 brother-sister pairs, themselves descending from about 100 parental families (F0). As expected, after genotyping, we found a minority of the F2 diploid males (120 diploid males vs 570 haploid males for wasps that were successfully genotyped), resulting in strongly unbalanced designs. We therefore discarded a number of haploid males in order to balance comparisons, and discarded a number of F1 families that produced no diploid male. While doing this, and to insure enough replicates for diploid males, we sometimes selected randomly two haploid and two diploid males from the same F1 pair. Moreover, the same individuals were sometimes used for different independent measurements. These data manipulations resulted in 26 diploid males and 24 haploid males from 16 F1 pairs compared for morphology, adult survival and mate-finding, and 31 diploid males and 33 haploid males from 17 F1 pairs compared for courtship, mating and sperm transfer. In the last experiment where males were tested in competition, we made no data selection and used a total 40 diploid and 179 haploid males from 40 different brother-sister F1 pairs.

Ploidy level (*N* versus 2*N*) of tested males was assessed from heterozygosity at microsatellite loci, with the assumptions that (i) males that are heterozygote at one or more loci are diploid, and (ii) the probability that a diploid individual is homozygote at 10 different microsatellite loci, and therefore misclassified, is small and negligible (given allelic frequencies in the population from which F0 individuals were selected, the probability of falsely classifying a true diploid male as a haploid was estimated at p=0.0023; C. Vayssade, in preparation). For this, the DNA of tested males (after they had been stocked in 96% ethanol at -20°C) was extracted from the thorax and abdomen using the commercial kit prepGeM (ZyGeM Ltd, Hamilton, New Zealand). Five dinucleotides (VC-001, VC-002, VC-068, VC-092 and VC-094) and five trinucleotides (VC-009, VC-036, VC-060, VC-066, and Vcan071) (C. Vayssade, A. Chuine, unpublished data, except for Vcan071, Mateo Leach, 2009) were amplified in a multiplex PCR reaction. Each reaction volume included 2µl of DNA, 5µl of 2X Qiagen Multiplex PCR kit (a buffer containing nucleotides and HotSstart Taq DNA polymerase) and forward and reverse primer at the final concentration of 0.1µM for markers VC-009, VC-036, VC-068 and VC-092, 0.2µM for markers VC-060, VC-066 and Vcan071, 0.4µM for marker VC-094 and 0.6µM for markers VC-001, VC-002. Forward primers were labeled with one of four different fluorochroms. The reaction volume was adjusted with ultrapure water. The PCR program consisted of 15 minutes at 95°C, followed by 25 cycles of 30 seconds at 94°C, 90 seconds at 58°C and 60 seconds at 72°C and finishing by final extension at 60°C for 30 minutes. PCR products were then added to 8.75µl of Hi-Di formamide and 0.25µl of GeneScan 500 LIZ Size Standard (Applied Biosystems Inc.) and loaded on an ABI 3130 sequencer (Applied Biosystems Inc.). Sample genotypes were scored using the GeneMarker program (version 1.75 SoftGenetics LLC, USA).

Behavioral observations were made with a pad running an event-recorder (The Observer, version 10.0; Noldus Information Technology, Wageningen, the Netherlands).

Statistical analyses of the data relied on generalized linear models (GLM), except when mentioned. GLMs were implemented with different distributions depending on data, and fitted by maximum likelihood estimation of the parameters associated with ploidy level as a recurrent explanatory variable. The significance of parameters was assessed by means of likelihood-ratio tests, the significance of which was evaluated with respect to χ^2 distributions. Data analyses were performed in the *R* statistical package (*R* Development Core Team 2011).

Morphology

Male body size was estimated via left and right hind tibia length. In *V. canescens* as well as most other insects, tibia length correlates with other morphometric measures (Pelosse *et al.*, 2007)

and is therefore considered a representative proxy of body size. Each hind tibia was measured three times under a microscope at ×4 magnification and the mean of the three repeated measurements was used in further statistical analyses. Male symmetry was estimated as the relative difference between left and right tibia length (the absolute value of the difference divided by left-right average size). This index departs from zero when the symmetry between left and right sides decreases. Male size and symmetry were analyzed using a GLM with a gamma distribution and an inverse link function.

Immature and adult survival

Although we did not assess the survival rates of immature stages directly, we tested the effect of ploidy level on survival rate by comparing the proportion of diploid males observed among males at emergence (before posterior selection of data) to the proportion expected theoretically in the case of brother-sister mating (i.e., 20%). If diploid males had a lower survival rate during larval development than haploid males, the proportion of diploid males among emerging males should be lower than expected. In order to estimate adult lifespan, we recorded the dates at which males emerged and died with a precision of 12 h (two daily observations). Between the two events, males were enclosed in a 70 x 10 mm plastic tube closed with a piece of cotton soaked with water at the top and honey at the bottom. Amount of water and honey were daily checked and replaced when necessary. Males for which adult lifespan was assessed had been tested in a wind tunnel just after emergence (see below). Because flight represents a significant energy expenditure (Amat *et al.*, 2012; Harrison & Roberts, 2000), only males that had flown during the two assays (97% of tested males) were used for the analysis of adult lifespan. We used a GLM with a Gamma distribution of errors, and an inverse link function

Mate-finding

Male orientation toward females was studied in a wind tunnel (described in Lo Pinto *et al.*, 2004; Metzger *et al.*, 2010b). Tests consisted in releasing males 60 cm downwind from a source of volatile sex attractants and characterizing upwind flight. The source was composed of 5 one-day old virgin females of *V. canescens* and 40 larvae of *E. kuehniella* enclosed into an open tube (5 cm diameter) laid horizontally and through which air was pushed with an additional pump. The flight chamber of the wind tunnel was 150 × 50 × 70 cm and the release platform were placed 25 cm from the floor of the chamber. Inside the flight chamber, light intensity was 4600 lux, airflow of 22 $cm.s^{-1}$, temperature 25°C and hygrometry 45±5 %RH. In addition, visual landmarks for takeoff and in-flight orientation (Vickers, 2000) were provided in the form of a false (carton) plant on which males

were released, two similar false plants placed beside the source, and pieces of colored paper randomly arranged on the four faces of the flight chamber.

Just before being tested in the tunnel, males were allowed to fly at least two hours in a large rearing cage placed inside the flight chamber. The test consisted of releasing each male individually on the takeoff plant in two consecutive trials. Male behavior was recorded in real-time from release to landing, or until 5 minutes had elapsed after release, whichever happened first. The source was considered attained if males, in at least one of the two trials, landed on the tube or flew more than 5 sec and less than 5 cm away from the tube. Every day, females and hosts used as sources of volatiles were replaced, the tube was cleaned, and the room containing the wind tunnel was thoroughly air-refreshed.

Each day across 18 consecutive days, 10 to 25 males were tested between 11:30 am and 04:30 pm. The takeoff latency and the flight duration before landing on the source were calculated from the raw behavioral recording, and analyzed for the first successful flight only. The proportion of males reaching the source was also analyzed. Males that did not fly during the two trials, or flew only once and missed the source, were excluded from the analysis.

Takeoff latency and flight duration (for males that reached the source) were analyzed via a GLM with a gamma distribution and an inverse link function. The proportion of males that reached the source was analyzed via logistic regression with quasi-binomial distribution to correct for over-dispersion (Crawley, 2007) and logit link function.

Courtship, mating and sperm transfer

Male ability to court, copulate and fertilize female gametes was studied in a simple setup. Each test consisted of enclosing a male and a genetically unrelated female in a plastic tube (70×30 mm) and observing sexual behaviors during 15 min or until mating had occurred, whichever happened first. This allowed estimation of the following variables: time before first courtship, mean courtship duration (over the different courtship sequences), time before mating, copulation duration, mating success, and number of male rejections by females.

After behavioral observations, each pair was kept in a petri dish for 24h under the same laboratory conditions, with food and water to promote mating (when it had not occurred within the 15 min of direct observation). Each female was then allowed to oviposit two hours on a patch of 40 second-instar *E. kuehniella* larvae and rearing medium, and then dissected in a solution of insect ringer under microscope at ×40 magnification. We assessed the success/failure of sperm transfer via the presence/absence of spermatozoids in the spermatheca and the presence/absence of females in

among offspring. Hence, each tested male could be characterized by its ability of court and mate a female 15 min upon encounter, by its ability to transfer sperm, and its ability to sire female offspring.

Each day across 17 consecutive days, ten to fifteen pairs were tested between 11:30 am and 04:30 pm. Additionnally, 17 one-day old virgin females from the mass-rearing followed the same treatment (including spermathecal inspection) except mate-encounter. These virgin females served to assess the effect of mating on offspring production.

Courtship duration, time before first courtship, time before mating, and copulation duration were analyzed via GLMs with Gamma distribution and an inverse link function. Mating success was analyzed via a logistic regression with quasi-binomial distribution and a logit link function. Numbers of male rejections by females were analyzed via a GLM with a quasi-Poisson distribution of errors and log link function. Sperm transfer was analyzed via a logistic regression with a quasi-binomial distribution and a logit link function. The number of daughters sired (as a fitness measure for male), as well as the total number of offspring, was analyzed via a GLM with a quasi-Poisson distribution and a log link function.

Male mating success in competition

The mating success of diploid males may depend on the intensity of competition with other males, especially haploid males. We therefore complemented the study of reproductive success with observations in population cages offering more space, and the possibility, for females, to choose among different males. Tested males were less than three days old and fed with honey and water.

Mating was observed in a 21 × 31 × 45 cm plexiglas cage between 11:00 am and 3:00 pm at 25±1 °C and 60±10 % RH. The day preceding a test, a pool of 16 to 20 virgin males were anaesthetized with CO2, and each male was marked with a unique combination of two water-paint dots. (Preliminary experiments where 59 females had each been exposed to 5 marked and 5 unmarked males showed that marked and unmarked males had the same mating success, suggesting that marking had no effect on mating behavior in V. canescens, as shown in an independent study on female foraging behavior; Desouhant *et al.*, 2003). Males were then released in the observation cage until the beginning of the test. A trial consisted of introducing a virgin, one-day-old female in the cage until mating was observed, or 15 min had elapsed, whichever happened first. When mating occurred, the female was recaptured and the successful male was identified. Each day, 10 females were tested with the same male mating pool, and the procedure was replicated on 13 different days (each day with a different male mating pool).

The ploidy of each male in each pool, whether they had mated or not, was obtained from individual genotypes. This yielded an estimation of the proportion of diploid males in each male mating pool to which females had been exposed. For each female, direct observation allowed to assess mating success, time before mating, and after genotyping, the ploidy of the successful partner. The presence/absence of spermatozoids in the spermatheca was also obtained from dissections.

Results

Overall, analyses of morphometric, life-history, and behavioral traits of *Venturia canescens* show that in most respects, diploid males are similar to haploid males. Diploid males are nonetheless slightly less successful than diploid males to mate with females, and contrary to haploid males, they sire no offspring.

Male individual differences

Adult size, measured via hind tibia length, was similar in haploid and diploid males (1435 ± 28 μm and 1462 ± 28 μm respectively; GLM, χ^2=0.005, *df*=1, *p*=0.49). Our index of symmetry based on the relative difference between left and right tibia length was also similar in the two types of males (0.01 ± 0.001 on average; GLM, χ^2=0.02, *df*=1, *p*=0.83). Diploid males were fully viable during immature and adult stages. Overall, brother-sister pairs produced 17% diploid males (80/470), a proportion that did not differ from the expected 20% (Chi-square test, χ^2=1.19, *df*=1, *p*=0.27). As adults, diploid males lived 16 ± 2.4 days (mean ± SE), which did not differ from the lifespan of haploids (18 ± 1.9 days; GLM, χ^2=0.09, *df*=1, *p*=0.68).

Male orientation toward odors of hosts and females was similar for haploid and diploid males. A similar proportion of diploid (18/31, 58%) and haploid males (23/33, 70%) reached the odor source in the wind tunnel (GLM, χ^2=0.94, *df*=1, *p*=0.34). Male ploidy did not influence the time spent before taking off from the release point (4.8 ± 1.2 s for diploid males vs 6.5 ± 3.6 s for haploid males; GLM, χ^2=1.43, *df*=1, *p*=0.63). Ploidy did not influence flight duration either (9.4 ± 3.1 s for diploids and 7.8 ± 1.2 s for haploids, respectively; GLM, χ^2=0.35, *df*=1, *p*=0.58).

During the 15 min of direct observation of courtship behavior, 81% of diploid males (21/26) and 96% of haploid males (23/24) engaged in courtship (Chi-square test, χ^2 =1.45, *df*=1, *p*=0.23). Neither mean courtship time nor time elapsed before first courtship (*i.e.* latency to court) differed significantly between haploid and diploid males (Table 1). Overall, one third of the tested males successfully mated with the female during the 15 min of observation, and although we found a

tendency for mating success being lower for diploids compared to haploids (19% vs 38%), mating success was statistically similar for the two types of males (Table 1). Females rejected haploid and diploid males indifferently (Table 1). What differed between haploid and diploid males was the time before mating (Table 1). Compared to a haploid male, it took four times longer for a diploid male to achieve a successful mating (Fig. 1), a result which did not depend on the time spent in copula which was similar for diploid and haploid males.

Table 1. Mating behavior of *Venturia canescens* males according to their ploidy (diploid or haploid). Mean value ± standard error, or proportions, is given for each behavioral item. χ^2 and p-values represent likelihood-ratio tests for the effect of male ploidy (one degree of freedom) derived from generalized linear models fitted to the data.

	Diploid	Haploid	χ^2	p-value
Time to 1st courtship (s)	40 ± 16	45 ± 18	0.20	0.82
Courtship duration (s)	12 ± 3	11 ± 2	0.07	0.79
Time to mate (s)	302 ± 99	71 ± 11	7.13	<0.001
Copulation duration (s)	170 ± 81	110 ± 19	0.63	0.29
Rejected males	19% (5/26)	29% (7/24)	0.68	0.42
Mating Success	19% (5/26)	38% (9/24)	2.08	0.15

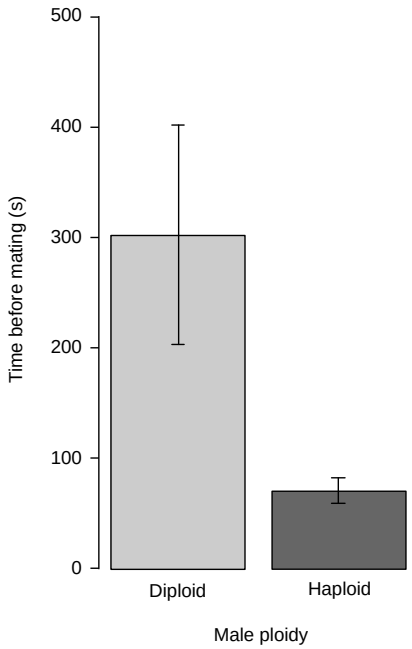

Figure 1. Mean time before mating for diploid and haploid males. Error bars are standard error of the means.

Both diploid and haploid males transferred sperm to females, as demonstrated by the presence of sperm in the spermatheca of females that had been exposed to males. After 24h, 41% of diploid males (7/17) and 61 % of haploid males (14/23) had mated with females and transferred sperm, proportions that did not differ (GLM, χ^2= 1.53, *df*=1, *p*=0.23). However, contrary to haploid males, diploid males sired no offspring: when exposed to 40 hosts during 2 h, females that had mated with a diploid male produced no daughter, whereas females mated with a haploid male produced an average of five daughters (Fig 2A). We nonetheless found one exception, with the presence of one triploid daughter in the progeny of a female mated by a diploid male. Males sired by mated females were all haploids, whether they had been mated by a haploid (subsample, N=129) or a diploid male (subsample, N=159). Hence, although diploid males resemble haploid males on all traits we measured, notwithstanding the difference in time before mating in individual tests, they appear unable to produce any offspring and have therefore no fitness.

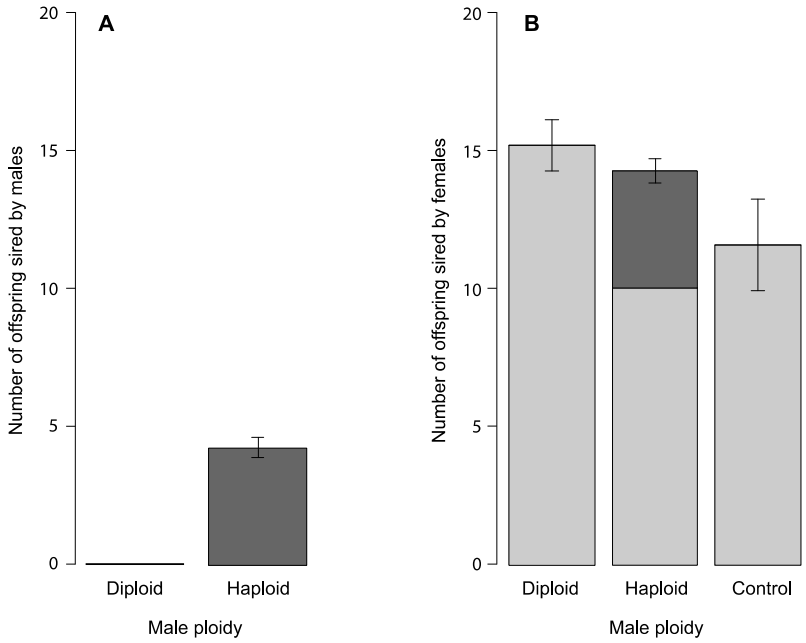

Figure 2. A. Mean numbers of offspring sired by diploid and haploid males **B.** Mean number of offspring sired by females that had mated with diploid or haploid males or had not mated (control). Light-gray bars represent sons and dark-gray represent daughters. Error bars are standard errors of the mean number of offspring.

Male mating success in competition

If mating was random with respect to male ploidy, we would expect the proportion of females mating with a diploid male to match the proportion of diploid males occurring in the mating cage. This is not what we observed. When pooled across all replicates (the 13 cages), the proportion of females that mated with a diploid male (0.10; 9/89) was about two-fold lower than the proportion of diploid males that occurred in the cages (0.19; 47/247; Fisher's Exact test, p=0.034). Hence, in competition, diploid males had a lower mating success than haploid males. Without pooling over replicates, we used a pairwise Wilcoxon-Mann-Whitney test to compare the proportion of females that mated with a diploid male to the proportion of diploid males that occurred in the cage (N=13). Again, we found a significant difference (V=10, p=0.014). We also found that the probability of mating with a diploid male was lower than the relative abundance of diploid males in 11 out of 13 replicates (Fig. 3), a ratio that would be unlikely if haploid and diploid males had the same mating success (11/13 has a probability p=0.011 under a binomial distribution with an expectation of 0.5).

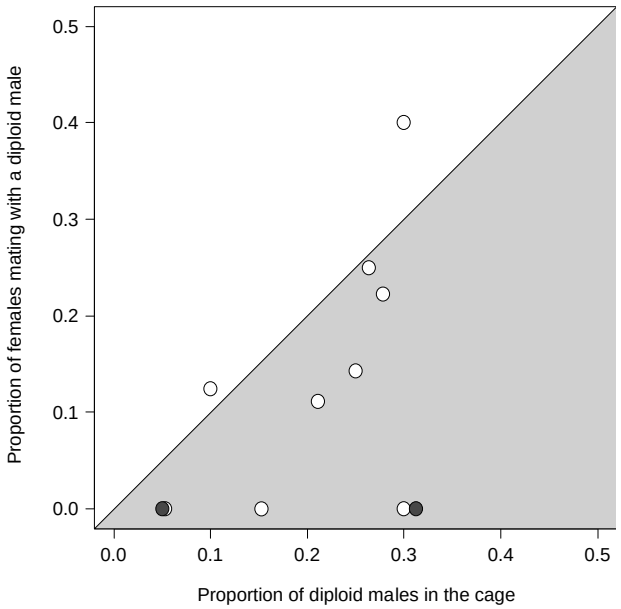

Figure 3. Proportion of females that mated with a diploid male versus proportion of diploid males occurring in the population cage (13 replicates). Each dot represents one (white dots) or two replicates (black dots). The light grey area indicates replicates for which the proportion of females mating with a diploid male is lower than the proportion of diploid males in the cage.

A non-negligible number of females (36 in a total of 129, that is, 30%) did not mate within 15 min of observation, but the probability of mating was unaffected by the proportion of diploid males occurring in the cage (mixed GLM with a binomial distribution and logit link function, and replicate as a random effect: χ^2= 0.80, *df*=1, *p*=0.37). The time before mating was neither affected by the proportion of diploid males in the cage (mixed GLM on log-transformed durations, with a Gaussian distribution and identity link function: χ^2= 0.37, *df*=1, *p*=0.54) nor by the ploidy of the successful males (χ^2= 1.58, *df*=1, *p*=0.21).

Modeling population consequences

Our mating experiment revealed that females that had mated with a haploid male or with a diploid male, and females that had not mated at all, produced the same total number of progeny (GLM, χ^2=9.9, *df*=2, *p*=0.14, Fig. 2B). What differed between female types was the offspring sex ratio. Females mated with a haploid male produced some daughters whereas females mated with a diploid male and virgin females produced no daughter but a similar number of sons (respectively 15 and 12 sons in four hours of exposition to hosts; GLM contrast matrix, χ^2=35.77, *df*=2, *p*=0.09). Hence, from the perspective of females, mating with a diploid male has the same consequence as

not mating at all. The production of all-male progeny by mated females is referred to as pseudo-virginity (Godfray, 1990).

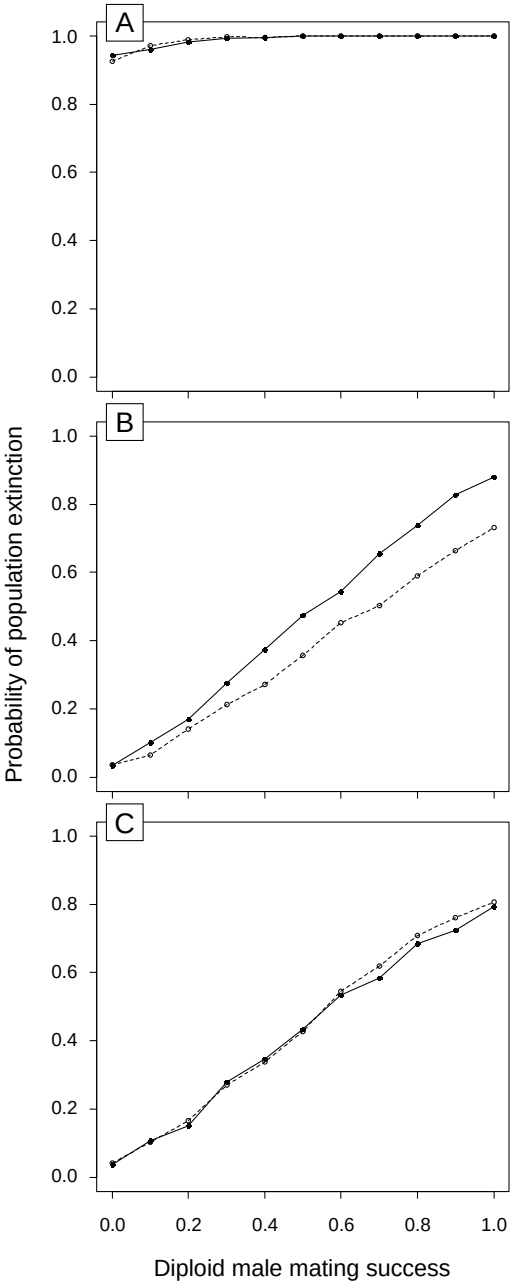

Figure 4. Probability of population extinction versus diploid male mating success for two alternative scenarios: females mated with diploid males sire unviable triploid offspring (empty circles, dotted lines) or females mated with diploid males are pseudo-virgin (filed circles and solid lines). Simulations were run with three contrasted combinations of parameter values for the environment carrying capacity (*K*) and the female net reproductive output (*NRO*, the expected number of offspring produced per female): (A) K = 500 and NRO = 2; (B) K = 50 and NRO = 10 and (C) K = 150 and NRO = 4.

In their original model, Zayed and Packer (2005) predicted the extinction probability of populations of haplodiploid organisms with sl-CSD, random mating, environmental and demographic stochasticity. Two contrasted scenarios for the fitness of diploid males were hypothesized: (1) unviability or (2) sterility, the latter resulting in the mortality of all eggs fertilized by sperm from diploid males (i.e., mortality of potential triploid daughters). When viable, diploid males were assumed as successful as haploid males to encounter and mate females. Hence, our results on *Venturia canescens* (along with observations on other species where diploid males trigger pseudo-virginity; Harpur *et al.*, 2013; Heimpel & de Boer, 2008) do not fit the modeling assumptions set by Zayed and Packer (2005).

To provide better and broader interpretations of our results, we developed a stochastic individual-based model assuming pseudo-virginity and varying diploid male mating success. For this, we revisited the modeling frame described in Zayed and Packer (2005) in order to contrast a scenario under which viable diploid males sire no offspring (pseudo-virginity) to a scenario under which viable diploid males sire unviable triploid females (mortality of potential daughters). In addition, we assumed that diploid males are not always successful to find and mate females, and ran sensitivity analyses to depict the consequences of diploid male mating success on population dynamics (our model is thoroughly described in Annex 1). For each scenario and different values for the mating probability of diploid males, 1000 simulations were run, each across 100 generations. Simulations were replicated for three different sets of parameter values for the environment carrying capacity (K) and the net reproductive output (NRO, the expected number of offspring per female). The probability of extinction P(E) was estimated as the proportion of simulations ending with population extinction. We also estimated the population sex ratio before extinction, with the expectation that a higher mating success of diploid males should result in a bias of the population sex ratio toward males.

Our simulations show that for different sets of parameters, the extinction probability increased with increasing mating success of diploid males (Fig. 4). When K was low and NRO was high (Fig. 4B), the probability of population extinction was slightly higher when diploid males triggered pseudo-virginity than when they sired triploid daughters. For other sets of parameters, the two scenarios yielded the same probability of extinction and a dramatic sensitivity to the mating success of diploid males (Fig. 4A and 4C). In all cases, the sex ratio before extinction was severely biased toward males.

Discussion

Single-locus complementary sex determination in insects of the order Hymenoptera results in a severe form of inbreeding depression underpinned by overdominance: homozygous individuals at the *csd* gene develop into males that are most often unviable or sterile (Cowan & Stahlhut, 2004; Harpur *et al.*, 2013; Heimpel & de Boer, 2008). Diploid males are thus a reproductive dead end. Nevertheless, subtle changes in some of their fitness components may have drastic consequences on the evolution of female behavioral responses to avoid producing – or mating with – diploid males, and on the dynamics of small populations. We discuss our findings in the light of these evolutionary and demographic perspectives.

In *Venturia canescens*, diploid males resemble haploid males in most respects. Despite the variety of morphological, behavioral, and life-history traits analyzed, we only found two significant differences between the two male types. First, when a single male was enclosed with a single virgin female, the time to mate was four times higher for diploid males than for haploid males, a delay that has also been observed in other species, such as *Cotesia rubecula* (de Boer *et al.*, 2012). Congruently, we found that in situations where diploid and haploid males compete for females and where females have the opportunity to chose their mate, diploid males had a lower mating success than haploid males. It is unclear whether these differences result from the discrimination against diploid males by females, or from independent attributes of diploid males. The similar probability of being rejected by females for each male type in the no-choice experiment suggests that the lower mating success of diploid males in the choice experiment is not a consequence of active female discrimination. This is however surprising because, as often observed in other parasitoid species (de Boer *et al.*, 2007; Harpur *et al.*, 2013), diploid male *V. canescens* do not appear less fit than haploid counterparts: for instance, they are of similar size, symmetry, and in the wind tunnel, they are as prompt as haploid males to takeoff toward females, and as fast when flying to reach them. The second difference between diploid and haploid males concerns fertilization success: although the two male types transfer apparently viable sperm to the female sperm storage organ (the spermatheca), sperm from diploid males do not fertilize any female oocyte (except once, as revealed by the occurrence of a triploid female among the offspring of a female that had mated with a diploid male). Our results show that in *V. canescens*, diploid males are viable but sterile and have a lower mating success than normal haploid males.

Whether diploid males are unviable or viable but sterile, each diploid male produced is analogous, for the parents, to the death of a female offspring. This major fitness cost has favored the evolution of inbreeding avoidance through protandry (Mazzi *et al.*, 2011), premating refractory period (Ode *et al.*, 1995), natal dispersal (Antolin & Strand, 1992; Gu & Dorn, 2003), or active mate choice (Metzger *et al.*, 2010a; Ode *et al.*, 1995; Thiel *et al.*, 2013). Yet, exceptions exist: diploid

males of some species are fully or partially fertile (Cowan & Stahlhut, 2004; de Boer *et al.*, 2007; Elagoze *et al.*, 1994; Elias *et al.*, 2009) and may therefore not trigger inbreeding avoidance (Stahlhut & Cowan, 2004). In *Venturia canescens*, females use volatiles to avoid mating with genetically related individuals (Metzger *et al.*, 2010a). Given the negligible level of inbreeding depression measured on other morphological and life history traits (Vayssade *et al.*, In press), inbreeding avoidance in *V. canescens* is best interpreted as an adaptive response to avoid the production of sterile diploid males.

Diploid males, when viable but sterile, may also represent a fitness cost to the female they mate with. Three different scenarios are generally delineated (Harpur *et al.*, 2013; Heimpel & de Boer, 2008): (1) Diploid males do not mate with females (e.g. Smith & Wallace, 1971); (2) diploid males mate with females, but because of the inability of unreduced diploid sperm to correctly fertilize female oocytes, females mated with a diploid male become constrained: they produce as many offspring as mated females, albeit only males (e.g. Holloway *et al.*, 1999); (3) diploid males mate with females, and diploid sperm fertilize oocytes, but sire triploid female offspring that are themselves unviable or sterile (e.g. de Boer *et al.*, 2007). Our findings on *Venturia canescens* fit the first and second scenarios: diploid males are as viable as haploids; they can mate with females, but are less successful than normal haploid males; diploid males are sterile, as shown by the male-only reproductive output of the females they inseminate. Hence, in *V. canescens*, females mated with diploid males are pseudo-virgin (Godfray, 1990), which means that they produce as many offspring as mated females, albeit only males. Equal fecundity of mated and virgin females is not a rule (Fauvergue *et al.*, 2008), but it holds for *V. canescens* (Metzger *et al.*, 2008), which comforts our findings.

In species such as *Venturia canescens*, the costs of mating with a diploid male should be analyzed via the theory on constrained oviposition and sex allocation (Godfray, 1990). In large, random-mating populations at sex ratio equilibrium, sons and daughters are strictly equal pathways for parents to produce grandchildren (Fisher, 1930). Thus, in such panmictic populations, mating with a diploid male and reproducing like a virgin has no cost. Wild populations of *V. canescens* resemble panmictic populations. First, as a consequence of host dispersion and weak rates of parasitism, most males and females emerge on different/distant patches (Driessen & Bernstein, 1999; Schneider *et al.*, 2003), and males search actively for females via a synergy of semiochemicals from females and hosts (Metzger *et al.*, 2010b). Second, genetic analyses of two distant field populations from southeastern France based on 19 microsatellite markers suggest no strong departure from Hardy-Weinberg equilibrium (C. Vayssade, A. Chuine and X. Fauvergue, unpublished data). Third, secondary sex ratios are weakly biased toward females as a possible evolutionary response to the long-term level of constrained oviposition (Metzger *et al.*, 2008) with the possible consequence of

maintaining the population sex ratio at Fisherian equilibrium (E. Desouhant, unpublished data). In such panmictic populations, females mated with a diploid male and producing only sons should have the same fitness as females mated with a haploid male and producing both sons and daughters.

The preceding conclusion, that mating with a diploid male has no cost, may not hold in small or isolated populations because at small population size, significant variations around sex ratio equilibrium favor mixed-sex broods (Taylor & Sauer, 1980; Verner, 1965). Consequently, pseudo-virginity, resulting from mating with a diploid male, becomes costly precisely when diploid males are expected more frequent. Over evolutionary times, the selective pressure on female discrimination against diploid males should therefore depend upon the frequency of population bottlenecks and the concurrent variations in the proportion of diploid males. Given the scarcity of robust data on the proportion of diploid males in the field (Heimpel & de Boer, 2008), discussing adaptive responses more thoroughly is perilous. In *Venturia canescens*, diploid males are rare in mainland populations (6%, 12/190, in the population of Nice, France) and possibly more frequent on islands (14%, 3/21 in the population of Majorca, Spain; A. Auguste and X. Fauvergue, unpublished data). In other species of parasitoid wasps, the proportion of diploid males was estimated around 10% in an introduced population of *Cotesia rubecula* (de Boer *et al.*, 2012) and 1% in a native population of *Bracon hebetor* (Antolin *et al.*, 2003). To some extent, these estimates corroborate the theoretical expectation that diploid males are infrequent because of balancing selection on sex alleles (Cook, 1993), and when combined with the relative harmlessness of diploid males when triggering pseudo-virginity, they may explain the apparent lack of discrimination by females.

In contrast, the mating ability of sterile diploid males may be much more dramatic at the ecological level. Extinction probability is expected much higher if sterile diploid males are fully capable of mating, because in this case, the decrease in net reproductive rate spans over two generations (Stouthamer *et al.*, 1992; Zayed & Packer, 2005). Our simulation model assumes that diploid males have varying mating success as a possible consequence of female choice. Whether mating with a diploid male triggers the mortality of fertilized eggs or pseudo-virginity yields the same relation between diploid male mating success and extinction probability.

More generally, our model shows the drastic consequences of individual behaviors such as male mating ability or female mate-choice on the extinction probability of small populations. Linking individual behavior to population dynamics is an exciting research avenue (Sutherland, 1996) to which host-parasitoid models have largely contributed (Bernstein, 2000; Cronin & Strong, 1999; Hassell & May, 1973; Hassell & May, 1985; Hassell & Varley, 1969; Hassell *et al.*, 1983). Most past approaches have nonetheless focused on behaviors promoting negative density-dependence and population stability. Our model contrasts with these classic studies: rather than seeking for processes improving demographic stability (the Holy Grail of host-parasitoid population

dynamics; Bernstein, 2000), we placed our research in the small population paradigm (Caughley, 1994) and non-equilibrium ecology (Rohde, 2005) by relating individual behaviors to population extinction and by including key genetic processes, as inbreeding depression, governing small populations. This focus on instability opens new frontiers to population biology and population management (Bompard *et al.*, 2013; Fauvergue, 2013; Fauvergue *et al.*, 2012; Hein *et al.*, 2009).

Acknowledgements

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Annex

We developed a simulation model to analyze how variations in the mating success of diploid males triggering pseudo-virginity affect the extinction probability of hymenopteran populations with single-locus complementary sex determination (sl-CSD). Our model is derived from that of Zayed & Packer {, 2005 #1054} and more specifically, the case where diploid males produce unviable triploid offspring. We added a novel scenario where diploid males induce pseudo-virginity in the female they mate with, as found in *Venturia canescens*. We also assumed that diploid males have a mating probability *p* that can differ from 1, either because they are less efficient than haploid counterparts at finding females, or because they are discriminated against by the female they encounter.

In each simulation run, the population started with an initial number of individuals, *N*, set at the environment carrying capacity, *K*, and a balanced sex ratio, as observed in *V. canescens* (Metzger, 2008). The initial number of alleles at the CSD locus, *n*, was determined according to the effective population size, *Ne*, and the mutation rate, μ, as proposed by Cornuet {, 1980 #4007}:

$$n = \sqrt{\frac{-2N_e}{\log(\mu\sqrt{8\pi}N_e)}}$$

Ne was set as a fraction of initial population size, i.e., *Ne* = 0.2 *K*, and μ at 10^{-6} mutations per generation. The *n* alleles at the *csd* locus were randomly assigned to each of the *K* founder individuals, with the assumption that the *K*/2 diploid individuals were heterozygotes, and therefore, female. At each generation, the following procedure was applied: (1) Males and females were paired at random, with the assumption that females mate only once whereas males possibly mate with several females. Diploid males have a mating probability of *p*. (2) For each female, the number of offspring depended on a constant expected net reproductive output (*NRO*), on which we applied variations among generations (environmental stochasticity) and among individuals (demographic stochasticity). For each female, the number of offspring was drawn from a normal distribution with mean *Xe* and variance *Vd* = 6 *NRO*, *Xe* being itself drawn at each generation from a normal distribution with mean *NRO* and variance *Ve* = *NRO*. (3) Each offspring could develop from a fertilized or an unfertilized egg, fertilization status being drawn from a binomial distribution with a mean of 0.5. (4) For each offspring, CSD alleles were assigned according standard Mendelian inheritance. (5) Offspring development into male or female depended on fertilization status, alleles at the CSD locus, and the hypothesized scenario. (6) If the total number of offspring outreached *K*, supernumerary individuals were randomly eliminated. (7) Offspring and their genotypes replaced their parents and parental genotypes, and the loop was run again until the 100^{th} generation.

One thousand replicated simulations were run for each of the two scenarios and a range of *p* values from 0 to 1 with a step of 0.1. We used three combinations of *K* and *NRO*: high *K* (500) and low *NRO* (2); low *K* (50) and high *NRO* (10) and intermediate values for *K* and *NRO* (*K* = 150 and *NRO* = 4). For each scenario and value of the *p* parameter, the probability of population extinction P(E) was calculated as the proportion of simulations ending with no individual.

CHAPITRE 5 : TEST DU « DIPLOID MALE VORTEX » DANS DES POPULATIONS EXPERIMENTALES

Nous avons recherché l'existence d'un effet Allee génétique et d'un effet Allee démographique (le « diploid male vortex » en cas d'effet Allee démographique fort) dans des populations expérimentales de *V. canescens* élevées en interaction avec leur hôte. Dans ces populations, seul le nombre initial d'hôtes était contrôlé. Ensuite, de la nourriture était apportée régulièrement aux hôtes, qui n'étaient plus manipulés. Les populations d'hôtes et de parasitoïdes se trouvaient donc dans des conditions propices au développement d'une dynamique hôte-parasitoïde, qui génère des goulots d'étranglement récurrent favorisant les extinctions. La diversité génétique dans les populations a été manipulée par trois traitements. Le nombre de familles fondatrices influence la diversité génétique initiale. La présence de flux de gènes et la capacité de charge manipulent la diversité génétique au cours du temps. D'après les modèles théoriques décrivant la dynamique de populations avec sl-CSD, on s'attend à ce que les populations avec moins de diversité génétique aient une plus proportion plus élevée de mâles diploïdes, un sex ratio plus biaisé vers les mâles, un taux d'accroissement réduit et une probabilité d'extinction plus élevée par rapport aux populations avec une diversité génétique plus élevée. Cependant, ces modèles simulent des populations dont la capacité de charge est constante, contrairement à ce qu'il se passe dans une dynamique hôte-parasitoïde. Les modèles n'incluent ni choix du partenaire, ni pseudo-virginité des femelles accouplées à un mâle diploïde, deux caractéristiques présentes chez *V. canescens* (Metzger *et al.* 2010, Chapitre 4). C'est pourquoi nous avons élaboré autre modèle individu-centré simulant l'expérimentation, afin d'obtenir des prédictions à confronter à celles des modèles précédents et aux résultats de l'expérimentation.

Comme les modèles précédents, ce modèle individu-centré prédit que les populations avec flux de gènes et capacité de charge élevée ont une meilleure probabilité de survie que les autres. Contrairement aux autres modèles, il ne prédit pas d'impact du nombre de fondateurs sur la probabilité de survie des populations. Dans les populations isolées simulées par le modèle, le nombre d'allèles au *csd* diminue au cours du temps. Au bout de 100 générations, les populations ont entre 3 et 6 allèles au gène du CSD, selon les modalités. Dans les populations simulées avec flux de gènes, de nouveaux allèles sont apportés régulièrement donc toutes les populations finissent par avoir le même nombre d'allèles (12-13). Dans les deux cas (populations isolées ou avec flux de gènes), le nombre initial d'allèles au *csd* n'est que faiblement corrélé au nombre d'allèles présent après plusieurs générations, qui est supposé déclencher le « diploid male vortex ». Le modèle prédit également que les populations avec un nombre élevé de fondateurs et/ou avec flux de gènes ont une

proportion de mâles diploïdes plus faible, ce qui est cohérent avec l'hypothèse du « diploid male vortex ». Cependant, une capacité de charge faible réduit légèrement la proportion de mâles diploïdes, contrairement aux prédictions des modèles précédents.

Certains résultats de l'expérimentation sont conformes aux prédictions d'au moins un des modèles. La diversité génétique aux marqueurs microsatellites, utilisée comme proxy de la diversité au gène du *csd*, était plus élevée dans les populations avec un nombre élevé de fondateurs et dans les populations avec flux de gènes. La proportion de mâles diploïdes augmentait au cours du temps et diminuait quand la diversité génétique augmente. La proportion de mâles diploïdes était plus faible dans les populations à capacité de charge faible que dans celles où la capacité de charge est élevée, et plus faible dans les populations à 10 fondatrices que dans les populations à 1 ou 2 fondatrices. Le sex ratio moyen était plus faible dans les populations avec un nombre élevé de fondateurs. Le temps avant extinction était plus élevé dans les populations à faible capacité de charge. Toutes les populations se sont éteintes sauf quatre.

D'autres résultats n'étaient conformes aux prédictions d'aucun modèle. La capacité de charge n'avait aucune influence sur la diversité génétique. Mais la diversité génétique a été mesurée (sur les 16 premières semaines) et elle n'avait peut-être pas encore été influencée par la capacité de charge des populations. La présence de flux de gènes n'influençait ni la proportion de mâles diploïdes ni le sex ratio, peut-être parce que le nombre d'individus transférés n'était pas assez élevé. Le sex ratio décroissait au cours du temps, ce que nous n'avons pas réussi à expliquer. Le taux d'accroissement n'était pas influencé par la diversité génétique initiale, ni par la proportion de mâles diploïdes ou le sex ratio. Les populations à capacité de charge faible avaient un taux d'accroissement plus élevé et s'éteignaient plus rapidement. Ceci peut être dû à la dynamique hôte-parasitoïde, qui génère des populations d'hôtes de petite taille. Plus les populations de parasitoïdes ont un taux d'accroissement élevé et plus elles susceptibles de parasiter tous les hôtes, entraînant à terme l'extinction de l'hôte et du parasitoïde. Le temps avec extinction n'était pas influencé par le nombre de fondateurs, ni par la présence de flux de gènes, ce qui suggère que la stochasticité démographique a un plus fort impact que le « diploid male vortex » sur la dynamique des populations.

Des analyses d'écart à la panmixie ont mis en évidence comportements d'évitement des accouplements entre apparentés, confirmant les comportements d'évitement des accouplements frère-sœur précédemment observés chez *V. canescens*.

Les résultats obtenus peuvent être expliqués par la présence de la première étape du « diploid male vortex » : les populations qui ont une diversité génétique plus faible produisent une proportion plus élevée de mâles diploïdes et les populations avec une proportion plus élevée de

mâles diploïdes ont un sex ratio plus biaisé vers les mâles, bien qu'aussi influencé par une autre variable non identifiée. Il semble donc qu'on observe les deux types d'effet Allee génétique décrits dans le chapitre précédent : dans les populations plus petites, les femelles ont moins de descendants fertiles (production de mâles diploïdes à la place de femelles) et un sex ratio plus biaisé vers les mâles. Cependant, comme les femelles *V. canescens* ont une fécondité élevée, il se peut que la réduction du nombre de femelles due à la production de mâles diploïdes soit compensée par une diminution de la compétition qui permet à chaque femelle d'avoir plus de descendants ; les femelles produiraient ainsi moins de descendants fertiles, mais ces descendants auraient une fitness plus élevée que sans production de mâles diploïdes. Les étapes suivantes du vortex d'extinction n'ont pas été détectées. La production de mâles diploïdes et les sex ratio n'influencent ni le taux d'accroissement ni la probabilité d'extinction. Les effets Allee génétiques dus à la production de mâles diploïdes ne se traduisent pas en effet Allee démographique. L'impact de la stochasticité démographique semble donc plus fort que celui de la production de mâles diploïdes, ce qui n'exclut pas que le « diploid male vortex » puisse être observé chez *V. canescens* dans d'autres conditions.

Article V

Testing the "diploid male vortex" in experimental populations of a parasitoid wasp: diploid male production does not enhance the probability of extinction

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Introduction

In small populations, demography and genetics can influence one another, creating extinction vortices: a decrease in population size initiates genetic deterioration (inbreeding depression, fixation of deleterious alleles, etc.), which decreases population size, and so on until population extinction (Gilpin and Soulé 1986; Tanaka 2000). Empirical demonstrations of extinction vortices are scarce. In a declining bird population, Blomqvist *et al.* (2010) showed a concomitant decline of heterozygosity and population size, with less heterozygous individuals having a lower fitness. More indirect evidences of extinction vortices were provided by Palomares *et al.* (2012) and Fagan & Holmes (2006). More demonstrations of extinction vortices would highlight the role of demogenetic interactions in the extinction of small populations.

In some species of Hymenoptera, the sex determination system may generate an extinction vortex (Zayed and Packer 2005). In Hymenoptera, males are haploid and females are diploid. In species with single-locus complementary sex determination (sl-CSD), haploids develop in males and diploids heterozygous at the sex determination locus (*csd*) develop in females. Diploids homozygous at the *csd* gene may also be produced and develop in diploid males, which are generally unviable or sterile. Consequently, populations with lower genetic diversity at the *csd* locus produce more diploid males. As diploid males are produced at the expense of females, these populations have a lower growth rate. If growth rate becomes negative, population size is reduced, increasing genetic drift and reducing the number of *csd* alleles, which leads to further decline in population size. This "diploid male vortex" was demonstrated theoretically in small populations with demographic and environmental stochasticity (Zayed and Packer 2005). The extinction vortex was stronger when diploid males mated and produced unviable offspring (Hein *et al.* 2009; Zayed and Packer 2005). Some individual behaviours like dispersal or mate choice reduced the probability of population extinction (Hein *et al.* 2009).

Beside these theoretical demonstrations, we lack empirical evidence for the diploid male vortex. Other processes than diploid male production can lead to population extinction (*e.g.* Allee effects, changing environmental conditions). To avoid observing these confounding effects, one can study experimental populations with controlled genetic diversity. The risk remains to confound the diploid male vortex with other cases of inbreeding depression or fixation of deleterious alleles. Measuring the proportion of diploid males circumvents this methodological problem. Four studies report the test of the diploid male vortex on experimental populations with controlled genetic diversity. In three species of social insects, the production of diploid males decreased the survival of colonies (Ross and Fletcher 1986; Tarpy and Page 2002; Whitehorn *et al.* 2009). However, a colony is not considered as a population because, in all three species, its members are the offspring of only one female. Moreover, all studies were conducted on one generation whereas the extinction vortex

is supposed to occur across several generations. The fourth study investigated the diploid male vortex across several generations, in experimental populations of the parasitoid *Cotesia glomerata* founded with different numbers of families. No evidence was found for an extinction vortex due to the production of diploid males: population size and fitness components decreased across generations but were not influenced by the number of founding families (Elias *et al.* 2010). The absence of diploid male vortex was explained by the fertility of *C. glomerata* diploid males and other forms of inbreeding depression with a higher impact on fitness than diploid male production.

Parasitoid populations regularly undergo bottlenecks due to their interaction with the dynamics of their host populations (Begon *et al.* 1995; Hassell 2000). This makes them particularly susceptible to the diploid male vortex. That is why we tested the diploid male vortex in experimental populations of *Venturia canescens*, a solitary parasitoid that attacks several species of pyralid Lepidoptera living in dried fruits (Driessen and Bernstein 1999; Salt 1976). In *V. canecens*, sex is determined by sl-CSD, leading to the production of fully viable but sterile diploid males (Beukeboom 2001). They can mate, although they have a lower mating success than haploid males (A. Auguste, unpublished data). Females are monandrous (Metzger, 2010); females mated with diploid males thus produce only males (A. Chuine, unpublished data). These females produce as many males as if they were virgin – they are pseudovirgin (A.Chuine, unpublished data). Females are reluctant to mate with brothers (Metzger *et al.* 2010), a possible adaptation to avoid producing diploid males. Under these conditions – monandrous species with sterile diploid males that mate –, theoretical models predict a strong negative impact of diploid male production on population dynamics (Zayed & Packer, 2005, Hein *et al.*, 2009, C. Vayssade, unpublished data).

Experimental populations were reared in interaction with their hosts. We only controlled the initial population size of hosts and parasitoids, after what we let host and parasitoid populations interacting without additional manipulations except regular provision of food for the host. We manipulated initial genetic diversity and applied other treatments that influence genetic diversity all along the experiment. Carrying capacity limits the maximal population size and, in turn, genetic diversity. Gene flow recurrently brings new alleles in the population. According to theoretical model predictions (Zayed & Packer, 2005, Hein *et al.*, 2009), we expected to observe an extinction vortex in the experimental populations. Populations with lower genetic diversity produce more diploid males, which increase the proportion of males in the population. Proportion of diploid males and sex ratio increase across time. Because of the production of diploid males, populations with lower genetic diversity have a lower growth rate and go extinct faster.

However, the models we refer to for predictions present some appreciable difference compared to the experiment. They considered populations with a fixed carrying capacity, whereas our experiment uses populations driven by host-parasitoid dynamics. Mate choice is absent from the

models, while *V. canescens* females avoid mating with their brothers and diploid males are less likely to mate than haploids. Finally, the scenario in which females mated with diploid males are pseudovirgin is not considered. That is why we created an individual-based model simulating the experiment, to confront its predictions to the ones from others models and to results from the experiment.

Materials and methods

Experimental design

We founded 96 experimental populations of the parasitoid *V. canescens* reared on the host *Ephestia kuehniella*. For the foundation of each population, hosts and parasitoids were introduced in a cage, after which no more insect entered the cage – except during the migration procedure for populations with gene flow – and both populations developed in interaction. We thus expected to observe a host-parasitoid dynamics. To create populations with different numbers of *csd* alleles, we applied three treatments in a fully crossed factorial design. First, we varied the number of founders per population. Parasitoid populations were founded with the progeny of one, two or ten pairs. The number of founding families should affect the initial number of *csd* alleles in the population. Secondly, the carrying capacity of the host population was controlled trough the initial number of hosts and the quantity of food supplied to the hosts. We used two values of carrying capacities, one being four-fold larger than the other. The carrying capacity of the host population influences the maximal size of host population, and in turn, the maximal size of the parasitoid population. Populations with low carrying capacity should thus be more affected by genetic drift, which could lead the loss of *csd* alleles. Thirdly, half of the populations recurrently received gene flow from other populations, while the other half remained isolated. Dispersal brings new alleles in the population and can maintain or increase the number of *csd* alleles. Eight replicated populations were founded for each of the 12 modalities obtained by crossing all treatments.

Individual-based model

To obtain predictions for our experiment, we built an individual-based model simulating the dynamics of interacting populations of *V. canescens* and *E. kuehniella* (Annex 1). The 12 modalities were simulated, with 100 repetitions per modality on a maximal duration of 100 weeks. The model included stochasticity on fecundity, developmental time and longevity for both hosts and parasitoids. The parasitoid population was considered extinct if no living or dead parasitoid was found during six consecutive weeks.

Foundation of experimental populations

To found experimental populations, 201 *V. canescens* females were captured in July to September 2011 on the Mont Boron, near Nice (France). This site includes a lot of carob trees, which are host plants for *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae), a natural host of *V. canescens*. To capture *V. canescens* females, open cups containing larvae of the host *Ephestia kuehniella* and semolina impregnated with attractive host kairomones were hanged in carob trees. Females were collected with a mouth aspirator while searching semolina. They were kept alive in closed plastic tubes and brought to the lab (temperature 24±1°C, photoperiod LD 16:8). During all the experiment, parasitoids were provided *ad libitum* with food (honey) and water. Each female from the field (generation G_0) was introduced in an individual cage (10 × 7 × 2.5 cm) containing about 50 second to fifth instar larvae of E. *kuehniella* reared in wheat semolina. After two days, the female was transferred to another similar individual cage containing the same number of larvae, for another period of two days, after what she was killed and preserved in 96% ethanol. Among emerging offspring (G_1), a maximum of 5 males and 5 females per family were collected within 30 minutes following emergence. As mating is scarce during this period, the individuals collected were presumed virgin. In total, 719 G1 males and 646 G1 females from 174 G0 different families were collected and kept in plastic tubes (Ø 4.8 cm, height 10 cm), with one tube par sex and family. According to their date of emergence, males and females were distributed in five groups, with a minimum of 100 males and 108 females from 44 different families per group. Each group was introduced in a mating cage (40 × 40 × 40 cm). After two days, females were taken out from the cage and introduced successively in two individual cages containing hosts, following the protocol described for G_0. Each G_1 family was randomly attributed one among the 12 modalities of the experiment. G_2 offspring were collected and kept in tubes as described for G_1 individuals. According to modalities, experimental populations were founded with one family (10 males and 10 females from the same family), two families (5 males and 5 females per family) or 10 families (1 male and 1 female per family).

Host populations were founded with 10 cohorts of host, each of a different age. Each week from nine weeks before foundation to population foundation, we prepared one box of hosts per population. We used two sizes of plastic petri-dish (Ø 5.7 or 2.8 cm, height 3.5 cm), for the two values of host carrying capacity (K). Boxes were filled with a one cm high layer of wheat semolina (19.5 g or 4.7 g of semolina). We added 6.7 or 1.6 mg of eggs of *E. kuehniella* (Biotop, Livron-sur-Drôme, France), which corresponds to about 60 and 260 eggs per box, respectively. Boxes were sealed with a piece of thin veil and maintained in a room at 27±1°C before their introduction in experimental population cages.

Each experimental population was maintained in a 21 × 31 × 45 cm cage. The floor was made of plastic, one large lateral wall of Plexiglas and one small lateral wall was a sleeve of cotton sheet closed by an elastic band. The three remaining walls were made of thin veil. In the center of the top wall, a circular hole enabled dispersion of parasitoids for populations of the "gene flow" treatment. A 15 cm long piece of plastic hose was inserted in the hole and closed at its lower extremity by a rubber plug. Honey and water were provided *ad libitum* during all the experiment. At population foundation, ten host boxes aged from 0 to 9 week were introduced into the cage along with the 20 parasitoids. We also founded eight populations containing only hosts (four populations for each level of K). The populations were randomly distributed among four climate-controled rearing rooms at 24±1°C.

Monitoring of experimental populations

Twice per week, dead host and parasitoid adults were counted, collected and preserved in 96% ethanol at 4°C. We separated male and female parasitoids, which are morphologically different. For some damaged individuals, sex could not be identified. Once per week, the oldest dish of semolina was removed with the host larvae it contained, if any, and replaced by a new dish of semolina. Once every four weeks, we applied the gene flow treatment to the half of populations (always the same populations at each gene flow session) by transferring parasitoids between populations. To transfer preferentially the individuals the most prone to disperse, we settled a trap by removing the plug of the hose and placed an Erlenmeyer upside down around the hose. This way, the individuals from the cage could enter in the Erlenmeyer through the hose. During the dispersal phase, for two consecutive days, we settled the traps and led them open for 8 hours each day. Every 30 minutes, we checked for the presence of parasitoids in the trap. When we found some, they were kept a plastic tube with food and water, using one tube per population and per sex. At the end of the trapping period, we transferred two males and two females from one cage to another. The plan of transfers was determined by random sampling of populations to form a sequence. Each population gave individuals to the following population in the sequence and received individuals from the preceding population. The sequence was changed at each gene flow session. When there were less than two males and/or two females in a population, we performed the transfer without changing the number of individuals in the population, *i.e.* if only one male and one female were present, they were transferred to the following population in the sequence, and replaced by one male and one female from the preceding population. It resulted in missing individuals in the following population and in too many individuals in the preceding population. This demographic change was corrected at the following transfer session.

Genetic analyses

Using 10 microsatellite markers, we performed genetic analyses on three groups: founder females, males and females from the experimental populations. First, we genotyped all the founder females (except three that escaped), and thus had, for each population, two third of the alleles of founders, the genotypes of founder males being unknown. For each population, we measured the number of alleles in the founder females, to check that the "number of founders" treatment indeed impacted initial genetic diversity. Secondly, genotypes of males gave measures of proportion of diploid males as well as genetic diversity within populations. Forty males per population were genotyped. These males were collected during the first 16 weeks of the experiment, which corresponds to about four generations. The first population extinctions occurred during the 16th week, thus we did not sample males after this date, to enable comparing all populations for proportion of diploid males and genetic diversity. As a measure of genetic diversity, we used allelic richness, computed with the FSTAT program. Thirdly, female genotypes were used to detect a departure for Hardy-Weinberg equilibrium resulting from mate choice behaviour. *V. canescens* females avoid mating with their brothers; we thus expected an excess of heterozygous females in the experimental populations, especially in small populations (low K), were encounters with brothers may be more frequent. To test this hypothesis, we genotyped 30 females per populations, in the modality expected to yield the highest (high K, gene flow and 10 founder females) and the lowest (low K, no gene flow and one founder female) genetic diversity. On Genepop version 4.2 (Rousset 2008), each population was tested for Hardy-Weinberg equilibrium with Hardy-Weinberg exact tests and Fis were calculated to detect a deficiency or excess of heterozygotes.

For genotyping, DNA of each individual was extracted using the commercial kit prepGeM (ZyGeM Ltd, Hamilton, New Zealand). Five dinucleotides (VC-001, VC-002, VC-068, VC-092 and VC-094) and five trinucleotides (VC-009, VC-036, VC-060, VC-066, and Vcan071) (C. Vayssade, A. Chuine, unpublished data, except for Vcan071, Mateo Leach, 2009) were amplified in a multiplex PCR reaction. Each reaction volume included 2µl of DNA, 5µl of 2X Qiagen Multiplex PCR kit (a buffer containing nucleotides and HotSstart Taq DNA polymerase) and forward and reverse primer at the final concentration of 0.1µM for markers VC-009, VC-036, VC-068 and VC-092, 0.2µM for markers VC-060, VC-066 and Vcan071, 0.4µM for marker VC-094 and 0.6µM for markers VC-001, VC-002. Forward primers were labeled with one of four different fluorochromes. The reaction volume was adjusted with ultrapure water. The PCR program consisted of 15 minutes at 95°C, followed by 25 cycles of 30 seconds at 94°C, 90 seconds at 58°C and 60 seconds at 72°C and finishing by final extension at 60°C for 30 minutes. PCR products were then added to 8.75µl of Hi-Di formamide and 0.25µl of GeneScan 500 LIZ Size Standard (Applied Biosystems Inc.) and

loaded on an ABI 3130 sequencer (Applied Biosystems Inc.). Sample genotypes were scored using the GeneMarker program (version 1.75 SoftGenetics LLC, USA).

Data analysis

Unless otherwise specified, we used generalized linear mixed models (GLMM) to analyse data. For all models, explanatory variables initially included the three treatments and their two-way interactions. Additional explanatory variables are detailed for each model. We used likelihood ratio tests to select of random effects. If no random effect was selected, a standard generalized linear model was built. Fixed effects were selected with the Akaike Information Criterion ($\Delta AIC = 2$). If several models had similar AIC values, we selected the model with the lowest number of explanatory variables. On the minimum adequate model, we tested the hypotheses of null value of coefficient for each fixed effect with type III Wald χ^2 tests. When tests revealed a significant effect for a factor with > 2 levels, least square means (LSM) were estimated for each level. Least-squares means were compared pairwise by Z tests and the p-values were adjusted using the Tukey method.

Initial genetic diversity was expected to be higher in populations founded with more females but to be unaffected by carrying capacity and gene flow because these treatments were applied after population foundation. To compare initial genetic diversity between the different treatment levels, we counted alleles at each locus for founder females of each population. We fitted a GLMM with a Poisson distribution of errors and a log link function. Identity of locus was treated as a random effect and the three treatments and their interactions, as fixed effects. With pairwise Wilcoxon signed rank tests, we then compared allelic richnesses of males collected in the first 16 weeks of the experiment among the different levels of each treatment. We used allelic richness at microsatellite loci as a proxy for allelic richness at the *csd* locus to test the hypothesis that populations with fewer founders, no gene flow and low carrying capacity have lower genetic diversity at the *csd* locus. The proportion of diploid males was expected to be higher in populations with lower genetic diversity. This variable was analysed using a binomial distribution of errors and a logit link function, with the population and the room of experiment as random effects and, as additional fixed effect, the time of collection of the male, mean allelic richness in the population and all the second-level interactions between the three treatments and time of collection. The sex ratio, defined as the proportion of males in the population, was analyzed with the same procedure, with the addition of the proportion of diploid males as fixed effect, with populations with higher proportions of diploid males and/or lower genetic diversity expected to display a more male-biased sex ratio. In order to use data representative from a majority of populations, analyses of sex ratio were performed only on data collected before half of the populations went extinct.

In parasitoid populations driven by host-parasitoid dynamics, parasitoid growth rate depends on the growth rate of the host, so that estimate the parasitoid intrinsic growth rate is tricky. That is why we used as a measure of parasitoid growth rate, the per capita searching efficiency *a*, defined by Nicholson & Bailey's (Nicholson and Bailey 1935) as the proportion of total hosts attacked by an individual. As males do not attack hosts, we expect *a* to decrease in populations with more male-biased sex ratios. We estimated values of *a* in each experimental population by fitting a host-parasitoid model to experimental data. The fitting procedure is described in Annex II. Estimated values of the searching efficiency *a* were expected to be lower in populations with male-biased sex ratio, high proportion of diploid males and/or low genetic diversity. It was analysed with a generalized linear model with a Gamma distribution of error and an inverse link function. As additional fixed effect, we include room of experiment, mean allelic richness, mean proportion of diploid males and mean sex ratio per population. A Cox proportional hazards function was used to analyse population times to extinction to test the hypothesis that populations with a low value of *a*, a male-biased sex ratio, a high proportion of diploid males, and/or low genetic diversity go extinct faster. The room of experiment was included as random effect and mean allelic richness, mean proportion of diploid males and mean sex ratio per population as additional fixed effects.

Analyses were conducted with the exactRankTests, lme4, glmulti, lsmeans, car and survival packages in the R statistical software. All values in the text are given as mean ± standard error of the mean (SEM).

Results

Predictions from the individual-based model

Predictions from the individual-based model are presented in Table 1, Figure 1 and Figure 2. Time before extinction of simulated populations was shorter with low K and shorter in isolated than in connected populations. These results are similar to the ones obtained by previous theoretical models simulating the dynamics of small populations with sl-CSD (Z&P, 2005, Hein *et al.*, 2009). The number of founders did not influence time to extinction (Fig. 1), contrary to predictions from Hein *et al.*'s model, in which populations initiated with more *csd* alleles had a lower extinction rate. Proportions of diploid males increased when number of founders decreased and were slightly higher in isolated than in connected populations. Unexpectedly, populations with low K had a slightly lower proportion of diploid males (Fig. 2). A model taking into account distinctive features of *V. canescens* biology thus provides some predictions that differ from the ones of more general models.

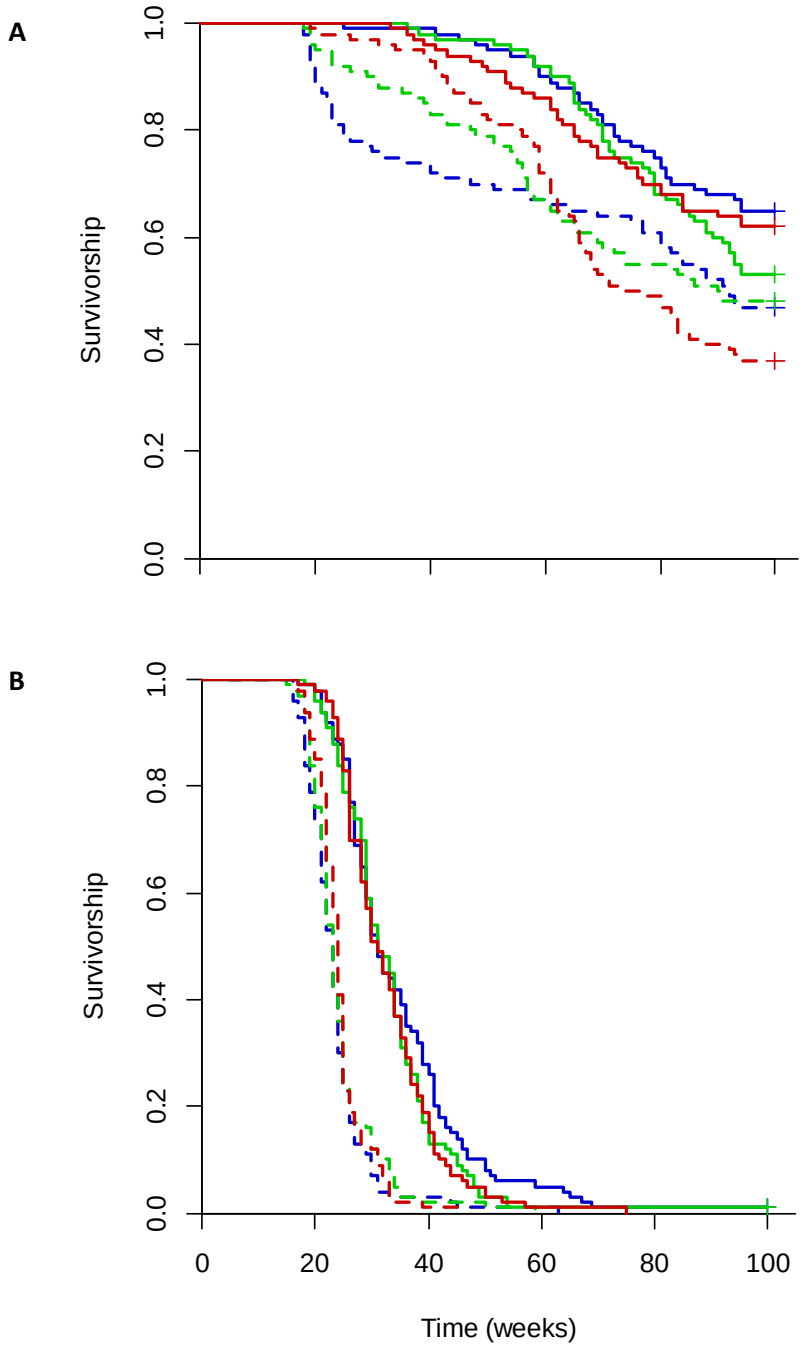

Figure 1: Survivorship curve of populations simulated with the individual-based model for populations with high (A) and low (B) value of K. Continuous lines represent connected populations and dashed lines, isolated populations. Blue, green and red lines represent populations founded with one, two and ten families, respectively.

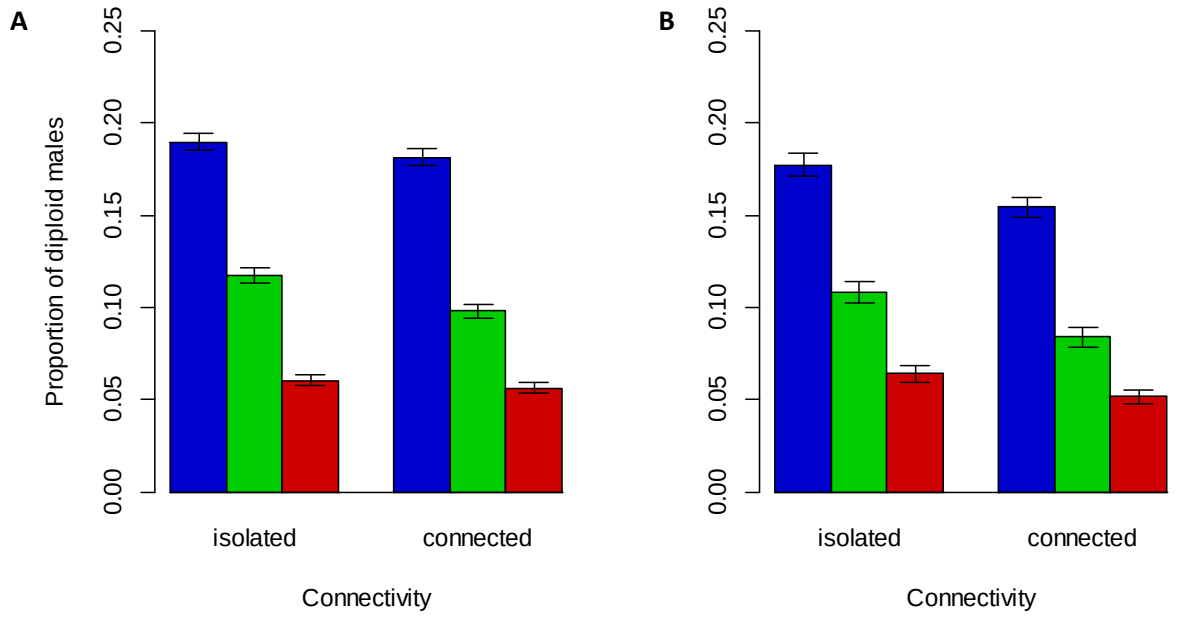

Figure 2: Mean proportions of diploid males among males in the first 16 generations of 100 populations simulated with the individual-based model for populations with high (A) and low (B) value of K. Blue, green and red lines represent populations founded with one, two and ten families, respectively. Error bars represent the mean ± standard error of the mean.

Initial genetic diversity

As intended, initial number of alleles per locus increased with the number of founder females (Tab. 2.A, LSM comparisons: 1 founder / 2 founders: z = -5.65, $p < 0.0001$; 1 founder / 10 founders: z = -10.51, $p < 0.0001$; 2 founders / 10 founders: z = -8.38, $p < 0.0001$). It was not influenced by other treatments, although statistical models including number of founders and K or gene flow had the same AIC as the selected model (Annex III.A).

Genetic diversity, production of diploid males and sex ratio

Allelic richness increased with the number of founder females (Fig. 3.A) and was higher for populations with gene flow than for isolated populations (Fig. 3.B), suggesting that these two treatment impacted genetic diversity in the expected way. However, allelic richness did not differ between the two levels of K (Fig. 3.C).

Table 1. Qualitative predictions of theoretical models simulating the dynamics of populations with sl-CSD and corresponding experimental results. "general theoretical models" refer to models by Zayed & Packer (2005) and Hein *et al.* (2009). "specific model" refers to the model created in this study and simulating the experiment. For genetic diversity, model predictions correspond to genetic diversity at the *csd* locus, while experimental results concern genetic diversity at microsatellite loci.

Variable	general theoretical models	specific theoretical model	Experimental results
A. Proportion of diploid males in 16 first weeks			
Number of founders	untested	-	-
Gene flow	untested	-	=
K	untested	+	+
Time	untested	+	+
Genetic diversity	untested	untested	-
C. Growth rate			
Number of founders	+	untested	=
Gene flow	+	untested	=
K	+	untested	-
Sex ratio	untested	untested	=
E. Time to extinction			
Number of founders	+	=	=
Gene flow	+	+	=
K	+	+	+
Growth rate	+	untested	=

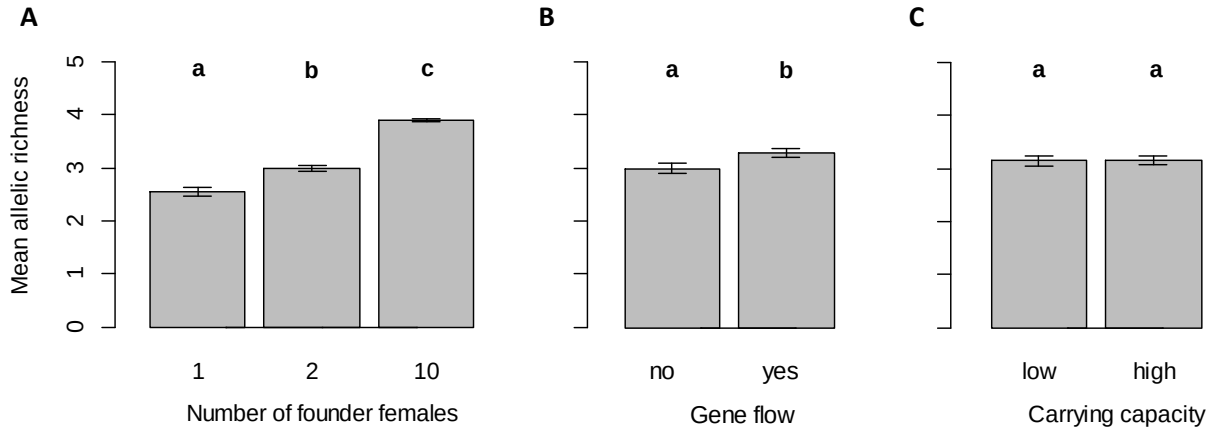

Figure 3: Mean allelic richness according to the three treatments: number of founder females (A), gene flow (B) and carrying capacity (C). Significant differences between levels are indicated by different letters.

Table 2: results of type III Wald χ^2 tests for all models selected. For each model are indicated the number of observations and the variance of random effects when present (L1 VC, L2 VC, L10 VC: variance of the locus identity random effect applied to populations with 1, 2 or 10 founder; P VC: variance of the population identity random effect).

Model	Df	χ^2	P-value
A. Number of alleles/locus in founder females, N =947, L1 VC = 0.003, L2 VC = 0.004, L10 VC = 0.047			
Number of founders	2	112.03	< 0.0001
B. Ploidy of male, N = 3738, P VC = 0.213			
Time	1	40.12	< 0.0001
K	1	9.63	0.0019
Allelic richness	1	20.75	< 0.0001
C. Ploidy of male, N = 3738, P VC = 0.194			
Time	1	40.82	< 0.0001
K	1	9.92	0.0016
Number of founders	2	22.95	< 0.0001
D. Sex ratio, N = 4073, P VC = 0.092			
Time	1	5.97	0.0146
Number of founders	2	1.85	0.3963
Time × Number of founders	2	49.96	< 0.0001
E. Estimate of searching efficiency, N = 96			
K	1	35.94	< 0.0001
F. Time to extinction, N = 96			
K	1	43.02	< 0.0001

Twenty-four statistical models were equivalent to analyse the proportion of diploid males and all of them included carrying capacity and time since population foundation (Annex III.B). Among them, we selected the two models with the lowest (three) number of explaining variables. In both models, the proportion of diploid males increased over time and populations with a high K had a higher proportion of diploid males than populations with a low K. The third explanatory variable was allelic richness in one model (Tab. 2.B), and the number of founder females in the other (Tab. 2.C). Populations with higher allelic richness had a lower proportion of diploid males (Fig. 4). The proportion of diploid males was lower in populations founded by ten females than in populations founded by one or two females but there was no difference in proportion of diploid males between populations founded by one and two females (Fig. 5). All results for diploid male proportion verify the predictions of at least one theoretical model simulating the dynamics of populations with sl-CSD (Tab. 1).

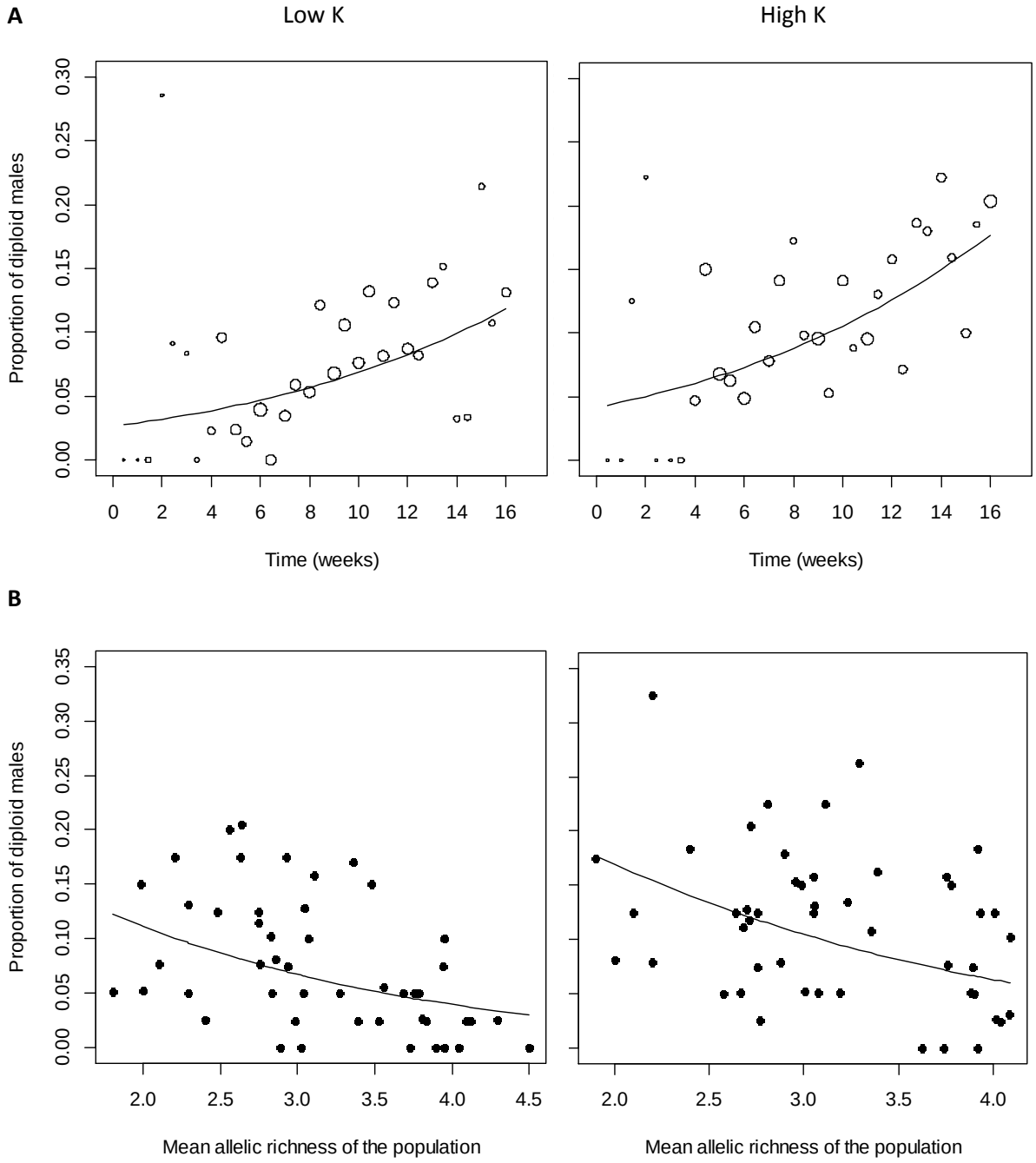

Figure 4: Proportion of diploid males as a function of time (A) and mean allelic richness of the population (B) in populations with low and high K. Lines represent predictions of the model calculated with the mean value of allelic richness (A) or time (B) for populations with low and high K. In the (A) graphs, the diameter of symbols is proportional to the number of males used to calculate the proportion of diploid males.

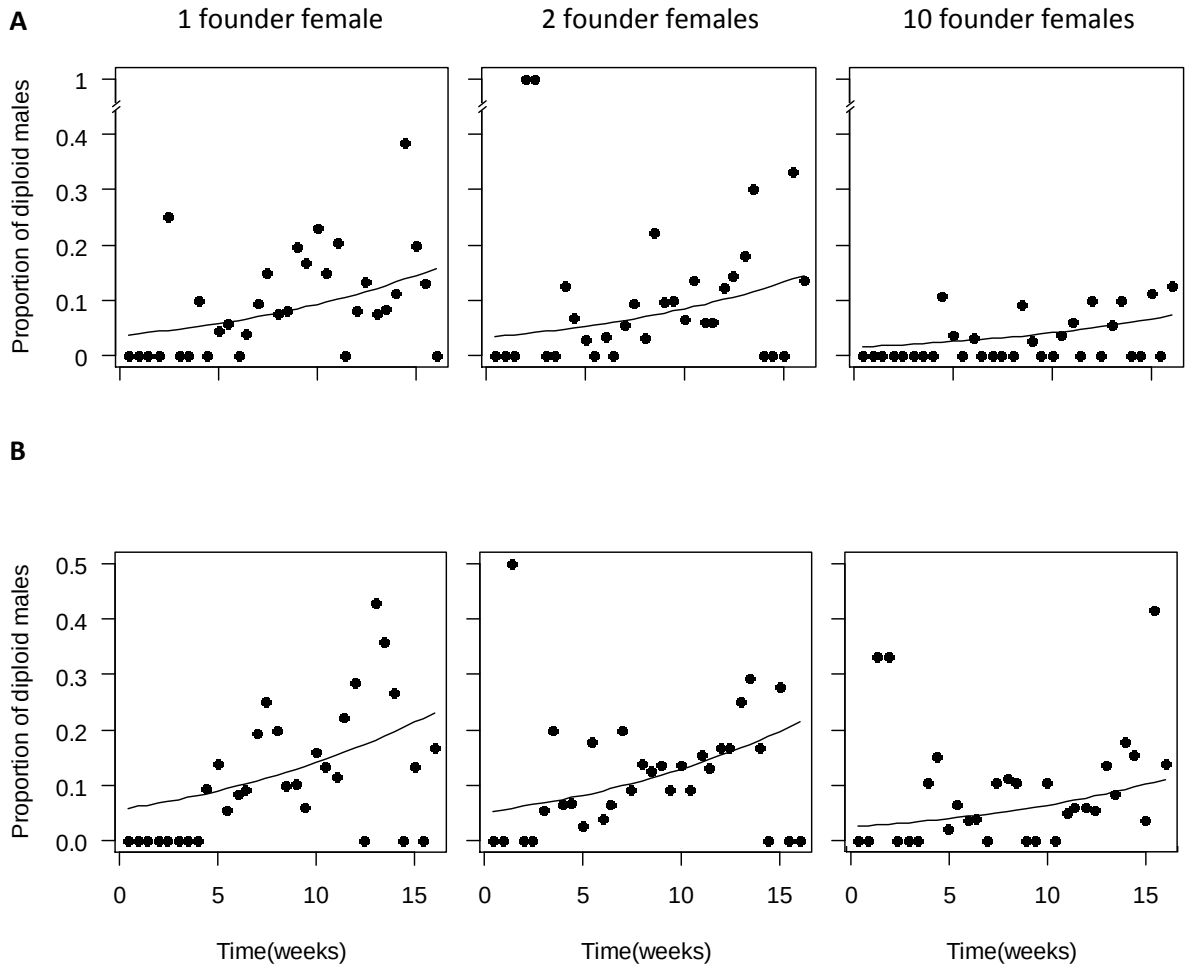

Figure 5: Proportion of diploid males over time in populations with large (A) and low (B) carrying capacity founded by 1, 2 or 10 females. Lines represent predictions of the model. The proportion of diploid males was lower in populations founded by 10 females than in populations founded by 1 or 2 females (1 founder / 10 founders: $z = 4.53$, $p < 0.0001$; 2 founders / 10 founders: $z = 3.39$, $p = 0.0002$). Populations founded by 1 and 2 female had similar proportions of diploid males ($z = -5.59$, $p = 0.8261$).

The sex ratio was affected by the time since population foundation and the interaction between time since foundation and number of founders (Tab 2.D). These two explanatory variables and the number of founders constituted the model with the lowest AIC, and were present in all equivalent models (Annex III.C). Contrary to model predictions (Tab. 1), the sex ratio decreased over time and this decrease was stronger in populations with a higher number of founder females (Fig. 6). However, the sex ratio data across time did not all resemble a linear function. For populations founded by one female, the decrease in sex ratio across time was low, with most values included between 0.55 and 0.75. For populations founded with two females, the evolution of sex ratio across time did not resemble a linear function, with values lower than 0.5 from the 2nd to the 7th week, then a peak around 0.6 followed by a decrease below 0.5 in weeks 10 to 15, and again an increase till about 0.6 (Fig. 6). The model simulating the experiment predicted a similar pattern for

sex ratio evolution across time. Only did the sex ratio of populations founded with ten females present a linear decrease, although it seemed to increase again around week 20 (Fig. 6). LSM values of sex ratio according to the number of founder females were different for the three numbers of founder females (1 founder / 2 founders: $z = 4.85$, $p < 0.0001$; 1 founder / 10 founders: $z = 7.39$, $p < 0.0001$; 2 founders / 10 founders: $z = 2.56$, $p = 0.0286$) and increased when the number of founders decreased, as predicted by theoretical models (Tab. 1).

In accordance with predictions, a higher number of founder females and the presence of gene flow increased genetic diversity. The proportion of diploid males increased across time and populations with lower genetic diversity or founded by fewer females had a higher proportion of diploid males. Mean sex ratio estimated with least-square means was higher in populations founded by fewer females. However, contrary to predictions, K did not influence genetic diversity or sex ratio, gene flow did not impact the proportion of diploid males nor the sex ratio and sex ratio decreased over time.

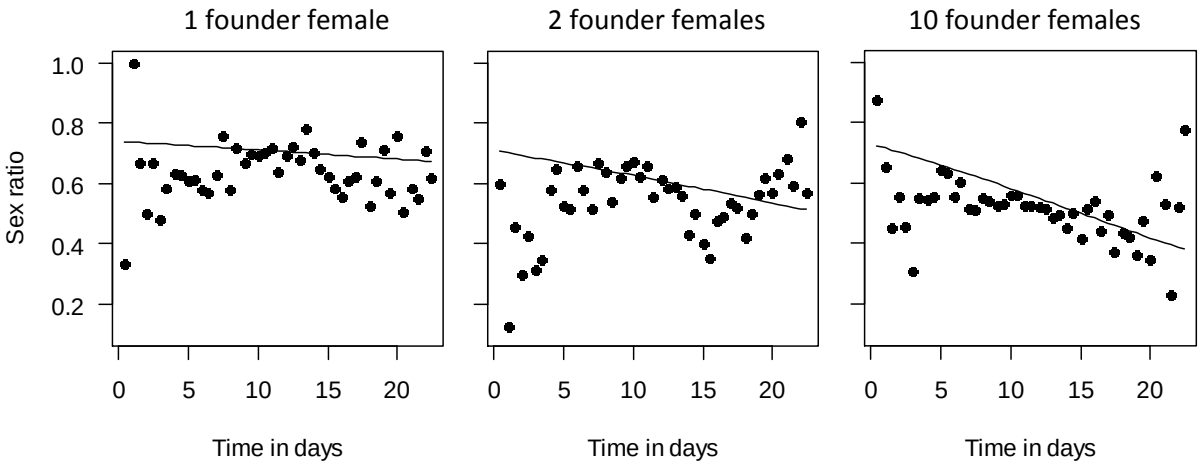

Figure 6: mean sex ratio (proportion of males) over time in populations founded with 1, 2 and 10 females. Lines represent predictions of the model. The proportion of males decreased faster with along time in populations founded by 10 females (1 founder / 10 founders: $z = 6.91$, $p < 0.0001$; 2 founders / 10 founders: $z = 4.23$, $p < 0.0001$), and faster in populations founded with 2 females than in populations founded with 1 female ($z = 3.00$, $p = 0.0076$).

Searching efficiency and probability of extinction

To analyse the estimate of mean searching efficiency within populations, the statistical model selected among six equivalent models included only K as explanatory variable (Tab. 2.E, Annex III.D): populations with low K had a much higher mean searching efficiency (0.1153 ±

0.0114) than populations with high K (0.0132 ± 0.0024). This is contrary to predictions from Z&P and Hein *et al.*'s theoretical models (Tab. 1), and was not tested in the theoretical model simulating the experiment. Probability of extinction also depended only on K in the model selected among 13 equivalent ones. Populations with low K went extinct faster than populations with high K (Tab 2.F, Annex 3.E). All populations went extinct except four with high K (Fig. 7). The effect of K on extinction probability was predicted by all theoretical models. However, predictions of the impact of number of founders and gene flow on probability of extinction were not verified (Tab. 1).

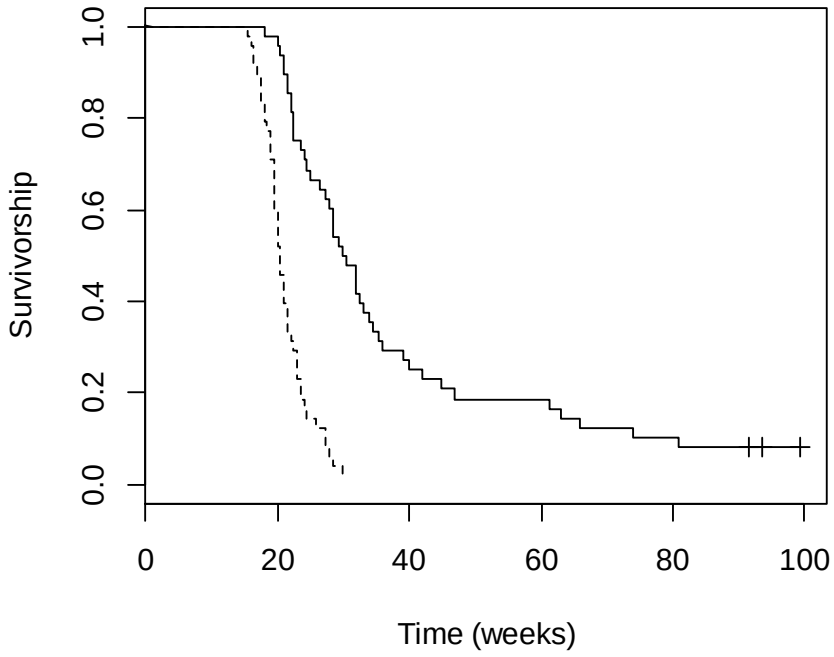

Figure 7: Survival curves of experimental populations. The dashed line represents populations with low K and the continuous line, populations with high K. Crosses indicate censored data.

Detection of mate choice behaviours

In 13 out of 16 populations, Fis value was negative, signalling a potential heterozygote excess (Tab. 2). All the eight populations expected to have the lowest genetic diversity (low carrying capacity, one founder female and no gene flow) had an Fis < -0.10. However, Hardy-Weinberg exact tests were significant in only 9 populations, among which 6 presented a heterozygote excess and 3 a homozygous excess (Tab. 3). These results are congruent with inbreeding avoidance mating behaviours described by (Metzger *et al.* 2010).

Table 3: Fis and results of Hardy-Weinberg exact tests for 16 populations: 8 in a modality with a high genetic diversity (high K, gene flow and 10 founder females: mean allelic richness 3.876 ± 0.087) and 8 in a modality with a low genetic diversity (low K, no gene flow and one founder female 2.268 ± 0.103).

Population	n	Fis	χ^2	Df	P-value
High K, gene flow, 10 founders					
Population 1	30	0.067	53.06	20	< **0.001**
Population 2	30	-0.067	26.90	20	0.138
Population 3	30	-0.076	36.44	20	**0.014**
Population 4	30	-0.099	24.35	20	0.227
Population 5	30	-0.065	37.37	20	**0.011**
Population 6	30	0.024	32.40	20	**0.039**
Population 7	30	0.023	31.63	20	**0.047**
Population 8	30	-0.096	Infinity	20	< **0.001**
Low K, no gene flow, 1 founder					
Population 9	30	-0.254	22.59	16	0.125
Population 10	27	-0.150	23.20	18	0.183
Population 11	21	-0.256	24.30	16	0.083
Population 12	30	-0.203	Infinity	14	< **0.001**
Population 13	30	-0.200	50.98	20	< **0.001**
Population 14	30	-0.203	27.21	12	**0.007**
Population 15	8	-0.420	12.36	14	0.578
Population 16	16	-0.102	13.77	20	0.842

Discussion

To test the diploid male vortex, we studied 96 experimental populations of the parasitoid *V. canescens*. Populations were reared under host-parasitoid dynamics, which supposedly generates recurrent bottlenecks. We manipulated three treatments that should have influenced initial genetic diversity (number of founding families) and recurrently reduce (carrying capacity) or increase (gene flow) genetic diversity. We followed populations across several generations and measured genetic diversity, proportion of diploid males, mean individual searching efficiency (an estimate of growth rate) and time to extinction.

We formulated predictions by creating an individual-based model simulating the experiment. Contrary to other, more general theoretical models, this model predicted no impact of the initial number of founders on time to population extinction. However, low carrying capacity and absence of gene flow decreased persistence probability, like in other models. This may be because

the number of *csd* alleles decreased across time and decreased more in populations with a higher initial number of alleles. After 100 generations, non-extinct isolated populations carried a mean of 3 to 6 alleles according to the number of founders. At the opposite, non-extinct populations with gene flow had about 12-13 alleles, whatever their number of founders. The absence of impact of the number of founders on population persistence may thus be due to a weak impact of initial genetic diversity on further genetic diversity. Gene flow, that recurrently increases genetic diversity, successfully lowers extinction probability.

Genetic diversity measured with microsatellite markers increased with the number of founding females and with gene flow but was not influenced by carrying capacity. As we analysed genetic diversity at the beginning of the experiment, carrying capacity may not have impacted genetic diversity yet.

The proportion of diploid males increased across time and when number of founders decreased, following the predictions of the individual-based model simulating the experiment and other theoretical models in which probability of population extinction increased in populations with fewer *csd* alleles (Hein *et al.*, 2009, Stouthamer *et al.*, 1992). However, contrary to model predictions, gene flow had no impact on proportion of diploid males, although it influenced neutral genetic diversity. Counter-intuitively, in both predictions from our model and in experimental populations, populations with low K had a lower proportion of diploid males but it was not due to a higher genetic diversity in low K populations. We can only say that, in the model, decreasing K decreased the proportion of diploid males without increasing the mean numbers of *csd* alleles. This effect of K remained when mate choice was removed (C. Vayssade, unpublished data).

The model fitted to sex ratio data predicted that the proportion of males was the highest at the beginning of the experiment and then, decreased across time and decreased faster in populations with more founders. However, observation of raw data nuances this conclusion because patterns of sex ratio evolution across time were not always linear (Fig. 6) and sometimes (populations with two founder females) resemble predictions from the model simulating the experiment. The decrease of sex ratio across time was not predicted by theoretical models and we do not know how to explain it. The stronger decline in sex ratio in populations with more founders led to different numbers of founders displaying different values for mean sex ratio, with higher sex ratios in populations with fewer founders. This fits the predictions of theoretical models, in which the higher production of diploid males in populations with fewer founders increases the sex ratio. In our experimental results, the higher production of diploid males in populations with fewer founders could explain that these populations have a higher sex ratio. Nevertheless, the increase across time of diploid male production is not coherent with the decrease of sex ratio across time. It thus seems that sex ratio is impacted by another variable than the production of diploid males.

Two of the treatments applied to experimental population – number of founders and gene flow – successfully impacted genetic diversity. This lead to the first step of the diploid male vortex: populations with lower genetic diversity produce more diploid males. The production of diploid males could explain that populations with fewer founders have a higher mean sex ratio, but not that their sex ratio decreased across time.

The further steps of the vortex were not detected in experimental populations. Production of diploid males and sex ratio did not impact population growth rate, estimated by the searching efficiency *a* of parasitoids, nor time to extinction. These two variables only depended on K. Populations with low K had a higher growth rate and went extinct faster. This is counter-intuitive and does not fit predictions by theoretical models for populations with stable carrying capacity. However, in populations driven by host-parasitoid dynamics, parasitoid populations with a higher growth rate attack more hosts and are thus more likely to eliminate the host population.

We have shown that populations founded by fewer individuals have a lower genetic diversity and a more male biased sex ratio, which can be partially due to the production of diploid males. A male biased sex ratio can represent a fitness cost for individuals: in a monandrous population with an excess of males, the mean fitness of males is lower than in a population with a balanced sex ratio because some males cannot mate (Taylor and Sauer 1980; Verner 1965). If competition between females is strong, each female lays fewer eggs than her maximal fecundity, because she does not encounter enough hosts to lay all her eggs. A decrease in the number of females, due to a more male biased sex ratio, enables each female to lay more eggs. The lower number of females is thus compensated by an increase in offspring production of each female, so that mean individual fitness does not change, though females have a higher mean fitness than males. Given the high fecundity of *V. canescens* females (about 75 adult offspring per female), it may be what happened in our experimental populations, and could explain that searching efficiency was not affected by the production of diploid males. Even in this situation, populations with male-biased sex ratio should be more affected by stochasticity on sex ratio: they have a higher probability to produce only males and thus to go extinct. This effect was not detected in experimental populations: populations with a higher sex ratio did not go extinct faster. It seems that stochasticity on sex ratio was negligible compared to demographic stochasticity. In natural populations of *V. canescens*, competition among females may be lower than in experimental populations (Driessen and Bernstein 1999). A more male biased sex ratio could thus lower natural population growth rates because it decreases the number of females without increasing their offspring production.

We investigated the presence of an extinction vortex, the diploid male vortex, in experimental populations of a parasitoid wasp. We demonstrated the presence of the first part of the vortex: populations with lower genetic diversity have a higher production of diploid males, which

may increase sex ratio, though other unknown variables influence sex ratio. The production of diploid males did not impact the growth rate of populations, which was only affected by the carrying capacity of the host population. As almost all populations went extinct anyway, they may have suffered strong demographic stochasticity that leaded them to extinction before the diploid male vortex appeared. These results comfort the hypothesis that very small populations are driven to extinction by demographic processes and not by genetic ones (Lande 1988), without excluding that larger populations may be affected by the production of diploid males.

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Annex 1: Description of the individual-based model

The model was used to simulate all 12 modalities of the experiment, with 100 repetitions per modality. One repetition lasted for 100 weeks or until the parasitoid population went extinct.

The model simulated an experimental population funded by mated females caught in a natural population with a maximum of 20 alleles at the CSD locus. All alleles had the same frequency, as expected under balancing selection (Yokoyama & Nei 1979). Before population foundation, the genotypes of founder females and males were created by random sampling of alleles. All founder males were haploid. Then, offspring of all founders were created. The genotype of haploid males at the CSD locus was created by sampling one of the two alleles of their mother. The genotype of females was the allele of their father and one of the two alleles of the mother. Diploid males were produced if a diploid offspring carried two copies of a CSD allele. In each family, the adequate numbers of male and female offspring were sampled to constitute a parasitoid population containing 10 males (some possibly diploid) and 10 females with equal contribution of all founding families. The host population was started with 10 cohorts of host larvae aged 0 to 9 weeks. The host population thus developed with overlapping generations. Each cohort had a carrying capacity of 180 unparasitized larvae or 285 parasitized larvae for populations with high carrying capacity, and 45 unparasitized larvae or 70 parasitized larvae for populations with low carrying capacity (adapted from Bernstein *et al.*, 2002). Hosts went through five age classes: egg, early-larva, mid-larva, late-larva and adult (Fig. I). Each adult host was characterized by its date of emergence, date of death and sex. For female hosts, mating status, egg load, date of death if virgin and date of death if mated were given. Parasitoids went through two age classes: larva and adult (Fig. I). Each parasitoid individual was characterized by its genotype at the CSD locus, name of its mother, date of emergence and date of death and belonged to one of three classes: females, haploid males and diploid males. In addition, females had a name, egg load and genotype of the male they mated with.

One time step corresponded to one week. At each time step, the following actions occured: (i) Mating of parasitoids at random. Females mated only once and males could mate several times. Each female had a 0.5 probability to reject a diploid male or a brother for mating. (ii) Dispersal in populations with gene flow. Two females and two males were sampled at random and replaced by two males and two females whose genotypes were determined by sampling alleles at random. (iii) Host survival to the late-larva stage. The probabilities for a host to survive the egg, early-lava and mid-larva stages were 0.85, 0.83 and 0.83, respectively (Lane & Mills, 2003). (iv) Parasitism. The proportion of late-larvae parasitized depended on the number and egg load of living female parasitoids. A minimum of 6.7% of larvae escaped parasitism (Bernstein *et al.*, 2002). (v) Survival to adult stage. The survival of hosts through the late-larva stage was density-dependent and given

by a Beverton-Holt equation whose parameters were determined for *V. canescens* in another study (Bernstein *et al.*, 2002). These parameters were different for parasitized and unparasitized larvae, the former having a higher probability to survive than the later, at high densities. (vi) Adult hosts. For each unparasitized host larva reaching adult stage, sex was determined by random sampling in a binomial distribution with mean 0.5. Mean developmental time was calculated as a logistic function of density of late-larvae (Lane & Mills, 2003). For each individual, a number was sampled in a Weibull distribution, multiplied by the mean developmental time, rounded and added to the current date to obtain the date of emergence. The date of death was obtained by a similar way: a number was sampled in a Weibull distribution, multiplied by the mean longevity, rounded and added to date of emergence. Parameters of the Weibull distribution and mean longevity depended on sex and female mating status. Egg load of each female was determined by random sampling in a normal distribution of mean 100 and standard deviation 75 (Lane & Mills, 2003). (vii) Adult parasitoids. Genotypes of parasitoids were generated from their parents ones' as previously described. The name of their mother was registered to enable sib-mating avoidance by females. Dates of emergence and date of death were determined each by a number randomly sampled from a Weibull distribution, multiplied by mean developmental time or mean longevity, rounded and added to the current date (for developmental time) or the date of emergence (for longevity). Mean longevity depended on the sex of the individual; females living slightly longer than males. Each female was attributed a unique name. Mean female egg load depended on late-larvae density. Each female egg load was driven from a normal distribution with the mean female egg load as mean and a standard deviation of 8.3 (Bernstein *et al.*, 2002). (viii) Host reproduction. For hosts, mating occurred as soon as both sexes were present. Mated females laid all their eggs just after mating. If they were older than one week, they laid only half their egg load.

At each time step, the model registered the number of individuals in each population and age class, numbers of dead hosts, parasitoid females, haploid males and diploid males, and number of CSD alleles. If no living or dead parasitoid was registered during six consecutive weeks, the parasitoid population was considered extinct and the simulation stopped. For each modality, we calculated the proportion of extinct populations, the mean time to extinction, the mean sizes of host and parasitoid populations, proportion of diploid males, sex ratio and number of alleles at the CSD locus.

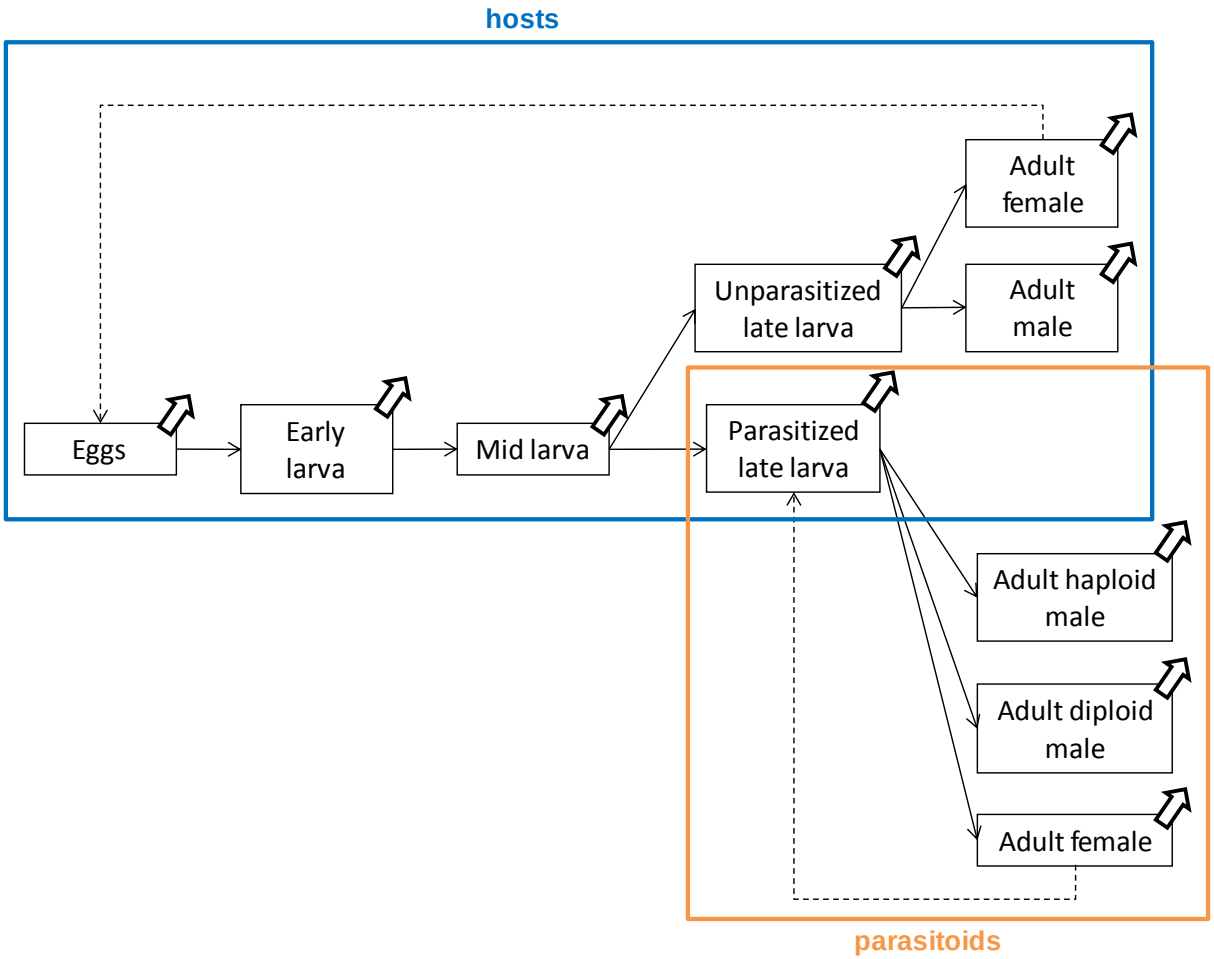

Figure I : Representation of the host and parasitoid life cycles in the individual-based model. Continuous arrows represent transition from one age class to another. Thick white arrows represent mortality during each stage. Dashed arrows represent egg laying.

Annex II : Estimation of the searching efficiency *a*

For each experimental parasitoid population, we estimated the searching efficiency *a*, used as an estimate of the intrinsic growth rate of the population. Several steps preceded this estimation. All mean values are given as mean ± standard error of the mean (SEM).

First, host-parasitoid models use abundances of living insects, while we only had counts of dead individuals. In a previous experiment with a similar protocol (D. Reiland & C. Vayssade, unpublished data), we had measured both numbers of living and dead individuals in experimental populations once per week. From these data, we computed cross-correlations between living and dead individuals for hosts and parasitoids. The correlation between dead (H_d) and living (H_l) hosts was maximal (0.785) for a lag of zero weeks. For parasitoids, the correlation was maximal (0.638) for a lag of two weeks. We thus used $H_{d(t)}$ and $P_{d(t+2)}$ as values for $H_{l(t)}$ and $P_{l(t)}$, respectively.

As generations are overlapping in the experiment, we fitted continuous time models to the data. All model fits were performed with nonlinear least-squares regressions. We expected numbers of hosts and parasitoids to influence one another: a high number of adult parasitoids should reduce the number of adult hosts at the following host generation and a high number of adult hosts should favour a high number of parasitoids at the following parasitoid generation. To use an appropriate model formula, we thus measured partial autocorrelation functions for hosts and parasitoids and cross-correlations between hosts and parasitoids in populations with and without parasitoids. For hosts, the mean values of the autocorrelation function were maximal for a lag of one week in both populations with (0.682 ± 0.012) and without parasitoids (0.605 ± 0.030). It was significantly different from 0 ($\alpha = 5\%$) in all 8 populations without parasitoids and in 96% (92/96) of populations with parasitoids. The autocorrelation function for the parasitoid population was maximal (0.342 ± 0.029) for a lag of 1 and it was significantly different from 0 in 49% (47/96) of populations. The mean value for the cross-correlation between numbers of hosts and parasitoids was maximal (0.367 ± 0.027) between $H_{(t-6)}$ and $P_{(t)}$ and was significantly different from 0 in 49% (47/96) of populations.

Then, we selected a model to describe the dynamics of the host. We fitted three models – logistic, Allee effect (Courchamp *et al.* 1999), Gilpin & Ayala (1973) – on host abundance in the 8 populations maintained without parasitoids. All models were of the form $\frac{dH}{dt} = rH_{(t)}G(H_{(t)})$, where *r* is the intrinsic growth rate of the hosts, $H_{(t)}$ the number of living adult hosts at time *t* and $G(H_{(t)})$ a density-dependent function that varied according to the model tested. Each model was fitted separately on populations with high and low K. $\frac{dH}{dt}$ was estimated by $H_{(t+1)} - H_{(t)}$. The logistic

model was the only one that could be successfully fitted to the data. Its parameter values were r = 0.229 (t = 4.68, p < 0.001) and K = 33.82 (t = 9.26, p < 0.001) for populations with high K and r = 0.133 (t = 2.02, p = 0.044) and K = 5.90 (t = 3.03, p = 0.003) for populations with low K.

The equations of the host-parasitoid model were:

(1) $$\frac{dH}{dt} = rH_{(t)}\left(1-\frac{N}{K}\right) - aH_{(t-5)}P_{(t-5)}$$

(2) $$\frac{dP}{dt} = aH_{(t-5)}P_{(t-5)} - d_pP_{(t)}$$

where $P_{(t)}$ is the number of living adult parasitoids at time t and d_p is the mortality rate of adult parasitoids. We calculated $\frac{dH}{dt}$ as $P_{(t+1)}$ - $P_{(t)}$. We used $H_{(t-5)}$ and $P_{(t-5)}$ so that the model generates a positive correlation between $H_{(t-5)}$ and $P_{(t+1)}$, as we had observed a positive correlation between $H_{(t-6)}$ and $P_{(t).}$ We first estimated a single value of d_p for all parasitoid populations because the adult mortality rate is density-independent (Bonsall & Hassel, 1998). From equation (2), a was expressed as a function of d_p. Then, this expression replaced a in equation (1), and the resulting equation was fitted to data to obtain an estimate of dp = 0.47 (t = 27.44, p < 0.001). With this value of d_p, for each parasitoid population, equation (2) was fitted to parasitoid data to estimate one value of a per population.

Annex III: For each variable analysed, list of all models with the lowest AIC. The model selected is written in bold. Random effects are indicated in brackets. Nb founders = number of founders.

Model	AIC
A. Number of alleles in founder females (locus ID)	
nb founders	**219.9**
nb founders + gene flow	221.1
nb founders + K	221.7
B. Proportion of diploid males (population ID)	
nb founders + K + time + K × nb founders	2237.1
nb founders + K + time	**2237.3**
K + time + allelic richness	**2237.9**
nb founders + K + time + allelic richness + K × nb founders	2238.0
nb founders + K + time + allelic richness	2238.0
nb founders + K + time + K × nb founders + K × time	2238.5
nb founders + gene flow + K + time + K × nb founders + gene flow × time + K × time	2238.7
nb founders + gene flow + K + time + K × nb founders + K × time	2238.7
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nb founders + gene flow + K + time + K × nb founders + gene flow × time	2238.7
nb founders + K + time + K × time	2238.8
nb founders + gene flow + K + time	2239.0
C. Sex ratio (population ID)	

nb founders + time + nb founders × time	**6281.4**
nb founders + gene flow + time + nb founders × time	6281.9
nb founders + gene flow + time + nb founders × time + gene flow × time	6281.9
nb founders + time + % diploid males + nb founders × time	6282.8
nb founders + K + time + nb founders × time	6283.4
nb founders + K + time + nb founders × time + K × time	6283.4
nb founders + time + allelic richness + nb founders × time	6283.4
D. Estimate of searching efficiency	
K + nb founders + K × nb founders	-436.89
K + nb founders + % diploid males + K × nb founders	-435.32
K + nb founders + allelic richness + K × nb founders	-435.28
K	**-435.1**
gene flow + K + nb founders + K × nb founders	-434.92
K + nb founders + sex ratio + K × nb founders	-434.89
E. Time to extinction	
K + searching efficiency	638.09
gene flow + K + searching efficiency	638.31
gene flow + K +allelic richness + searching efficiency	638.34
nb founders + gene flow + K + searching efficiency	638.6
nb founders + K + searching efficiency	638.71
K + allelic richness + searching efficiency	638.8
nb founders + gene flow + K + searching efficiency + gene flow × nb founders	639.65
K	**639.7**
nb founders + gene flow + K +allelic richness + searching efficiency + gene flow × nb founders	639.73
gene flow + K + searching efficiency + K × gene flow	639.81
gene flow + K + allelic richness + searching efficiency + K × gene flow	639.87
K + % diploid males + searching efficiency	639.89
K + searching efficiency + sex ratio	640.09

CHAPITRE 6 : DISCUSSION GENERALE

6.1 Principaux résultats

Cette thèse comporte deux objectifs. Le premier est d'encourager les collaborations entre généticiens et dynamiciens des populations en proposant un concept qui englobe des processus génétiques et démographiques. Nous avons choisi de définir et décrire l'effet Allee génétique. Le deuxième objectif est de rechercher l'existence d'un effet Allee génétique et de ses éventuelles conséquences démographiques dans des populations expérimentales de *V. canescens*, un Hyménoptère dont le système de détermination du sexe entraîne la production de mâles stériles dans les populations dont la diversité génétique est faible.

Pour répondre au premier objectif, nous avons proposé une définition des effets Allee génétiques. Un effet Allee génétique est un effet Allee élémentaire qui apparaît quand deux conditions sont réunies. La première condition est une relation entre la taille de population et un paramètre de la structure génétique (ex : hétérozygotie, richesse allélique). La deuxième condition est une relation entre ce paramètre de la structure génétique et une composante de la fitness. Pour qu'un effet Allee génétique soit observé, la direction des relations (positive ou négative) dans les deux conditions doit être telle qu'une réduction de la taille de la population entraîne une baisse d'une composante de la fitness. Une recherche bibliographique nous a permis de détecter 15 études décrivant un effet Allee génétiques, dont la plupart ne mentionnent pas le terme « effet Allee ». Les résultats de la recherche bibliographique suggèrent qu'il existe de nombreux autres cas d'effets Allee génétiques, qui n'ont été décrits moins précisément que les exemples cités dans l'article I (*e.g.* études citées par Leimu *et al.* 2006). Nous avons donc jugé important d'inclure dans l'article des conseils pour détecter un effet Allee génétique, en recommandant la création de populations expérimentales dont on peut manipuler la taille et la diversité génétique indépendamment l'une de l'autre. La dépression de consanguinité, le fardeau de dérive et le fardeau de migration sont les trois mécanismes impliqués dans l'apparition d'effets Allee génétiques. Nous rapportons deux cas d'effets Allee démographiques générés par un effet Allee génétique. Un effet Allee démographique d'origine génétique est aussi suspecté dans 5 autres études (citer) dont liste n'est pas exhaustive. Les effets Allee génétiques joueraient donc un rôle non négligeable dans l'apparition d'effets Allee démographiques car seule une quinzaine de cas d'effet Allee démographique pour lequel on connait le mécanisme de l'effet Allee élémentaire ont été mentionné dans la review de Kramer *et al.* (2009). Un effet Allee démographique dû à un effet Allee génétique

correspond à une démographie-génétique-démographie qui peut initier un vortex d'extinction s'il s'agit d'un effet Allee fort.

Plusieurs travaux préparatoires ont été effectués avant de commencer les expérimentations visant à répondre au 2ème objectif : tester expérimentalement la présence d'un effet Allee génétique, et éventuellement démographique, dû à la production de mâles diploïdes chez *V. canescens*.

Nous avons mis au point des marqueurs microsatellites qui permettent de mesurer à la fois la ploïdie des mâles et des paramètres de génétique des populations. Dans les expériences suivantes, nous utiliserons la diversité de ces marqueurs comme un proxy de la diversité génétique au gène du *csd*.

Nous avons recherché la présence d'un effet Allee génétique dû à la production de mâles diploïdes dans des populations naturelles et captives. Les populations isolées et/ou goulotées avaient une diversité génétique plus faible aux marqueurs microsatellites et une proportion de mâles diploïdes plus élevée. On peut donc supposer que ces populations subissent un effet Allee génétique : dans les populations plus petites les femelles produisent moins de descendants fertiles.

La dépression de consanguinité a été mesurée chez femelles des femelles de *V. canescens*. Un seul trait, charge en œufs à l'émergence, est affecté par la dépression de consanguinité. La charge en œufs à la mort ne l'est pas. Même dans l'hypothèse où la charge en œufs resterait plus faible pour les femelles consanguines tout au long de leur vie, la production d'œufs par les femelles consanguines reste élevée (charge de 100 œufs à la mort sans ponte). En situation de compétition, les femelles sont limitées par le nombre d'hôtes disponibles, et pas par leur charge en œufs. Le nombre d'hôtes parasités sera donc le même, que les femelles soient consanguines ou non. Par contre, dans les populations naturelles, il se peut que la compétition soit plus faible, auquel cas les femelles consanguines auraient une fécondité plus faible que les autres. Enfin, on ne peut pas exclure que d'autres traits, que nous n'avons pas mesurés, soient affectés par la dépression de consanguinité.

Nous avons ensuite mesuré la fitness des mâles diploïdes et ses conséquences pour la population. Les mâles diploïdes sont similaires aux mâles haploïdes mais ont une probabilité d'accouplement plus faible et sont stériles. Les femelles accouplées à mâles diploïdes sont pseudo-vierges : elles produisent autant de descendants que les femelles accouplées, mais uniquement des mâles. La production de mâles diploïdes peut générer deux types d'effet Allee génétique. Le premier concerne les femelles mères des mâles diploïdes et est présent dans toutes les populations quand la taille de population diminue, le nombre de descendants fertiles produit par femelle diminue. Le deuxième effet Allee génétique concerne les femelles accouplées avec des mâles

diploïdes dans des petites populations où le sex ratio est biaisé vers les mâles. Dans ce cas, les mâles étant plus nombreux que les femelles, ils ont une probabilité d'accouplement, et donc une fitness, réduite. D'après les résultats de simulations d'un modèle théorique, une plus faible probabilité d'accouplement des mâles diploïdes réduit la probabilité d'extinction. Sous certaines conditions (faible capacité de charge et taux d'accroissement élevé, comme dans les populations de l'expérience suivante), les populations avec femelles pseudo-vierges subissent plus d'extinctions que les populations dans lesquelles les femelles accouplées à des mâles diploïdes produisent des descendants triploïdes non viables.

Dans des populations expérimentales de *V. canescens*, nous avons recherché la présence d'un effet Allee génétique, donc élémentaire, et d'un effet Allee démographique dus à la production de mâles diploïdes. Nous avons créé des populations expérimentales de *V. canescens* avec différents niveaux de diversité génétique initiale (manipulée *via* le nombre de fondateurs) et au cours du temps (manipulée *via* la présence ou l'absence de flux de gènes et la capacité de charge). Ces populations ont été élevées en interaction avec celles de leur hôte de façon à favoriser la mise en place d'une dynamique hôte-parasitoïde et donc de goulots d'étranglement récurrents. Les populations ont été suivies sur plusieurs générations. Les deux effets Allee génétiques précédemment décrits ont été détectés : les populations dont la diversité génétique est faible ont une proportion plus élevée de mâles diploïdes (donc moins de descendants fertiles) et un sex ratio plus biaisé vers les mâles (donc une plus faible probabilité d'accouplement des mâles). Par contre, aucun effet Allee démographiques n'a été détecté : le taux d'accroissement et la probabilité d'extinction ne sont pas influencés par la production de mâles diploïdes. Les extinctions observées, qui concernent presque toutes els populations, seraient donc surtout due à la stochasticité démographique.

6.2. Intérêts et limites de l'expérience de test du vortex

Cette section discute de l'analyse des données, de la détection des effets Allee génétiques et de l'identification des causes d'extinction dans l'expérience de test du « diploid male vortex ».

Pour cette expérience, nous avons choisi d'élever les populations dans des conditions favorisant l'apparition d'une dynamique hôte-parasitoïde. Cette dynamique créé des goulots d'étranglement, ce qui devrait favoriser la perte d'allèles au gène du CSD et l'apparition du « diploid male vortex ». De plus, cette situation mime une situation de lutte biologique, dont le but est que la population de parasitoïdes auxiliaires ait un fort impact sur la démographie de la population d'hôtes. C'est la première fois que le « diploid male vortex » est testé dans une

population en interaction avec celle de son hôte, l'autre étude abordant ce sujet chez un parasitoïde avait utilisé des populations à capacité de charge fixe.

L'utilisation de populations dont la capacité de charge varie en fonction de l'effectif de la population d'hôtes rend toutefois l'analyse des données plus complexe. Comme le taux d'accroissement de la population de parasitoïdes dépend du nombre d'hôtes disponibles, qui varie au cours du temps, la mesure du taux d'accroissement de la population de parasitoïdes est plus complexe que dans une population seule élevée avec une quantité de ressources constante. Nous avons utilisé une estimation de l'efficacité de recherche du parasitoïde comme mesure du taux d'accroissement des populations. Pour cela, nous avons ajusté un modèle de dynamique hôte-parasitoïde à nos données, ce qui nécessitait auparavant d'estimer plusieurs variables par ajustement de modèles de dynamique des populations. Notre mesure du taux d'accroissement des populations est donc moins directe qu'elle l'aurait été dans une population de capacité de charge contrôlée et constante. Les hôtes étaient élevés en génération chevauchante, afin qu'il y ait toujours des hôtes disponibles pour les parasitoïdes dans la population (Le cycle de vie de l'hôte est plus long que celui du parasitoïde). De ce fait, les populations de parasitoïdes se développaient elles aussi en générations chevauchantes. Or, la plupart des modèles de dynamique hôte-parasitoïde considèrent des populations avec générations discrètes, ce qui a restreint notre choix de modèles à ajuster aux données. Enfin, l'interaction hôte-parasitoïde peut constituer une source d'extinction autre que la production de mâles diploïdes : si les parasitoïdes éliminent tous les hôtes, ou si la population d'hôtes s'éteint par stochasticité démographique à cause de sa petite taille, la population de parasitoïdes s'éteindra aussi, sans que son extinction ne soit due à la production de mâles diploïdes, ce qui limite la probabilité d'observer le « diploid male vortex ». Il se pourrait néanmoins qu'une population comportant plus de mâles diploïdes et donc moins de femelles ait un impact moindre sur la population d'hôtes et favorise sa propre survie. C'est ce qu'ont montré, pour certaines valeurs de paramètres (efficacité de recherche et taux d'accroissement de l'hôte élevés), les modèles théoriques d'A. Bompard, I. Amat, X. Fauvergue et T. Spataro. Ce résultat à l'opposé des prédictions des modèles sans dynamique hôte-parasitoïde souligne l'intérêt d'étudier des populations soumises à cette dynamique. Ce cas de production de mâles diploïdes favorisant la persistance de la population n'a pas été mis en évidence dans notre expérimentation, qui, d'après les valeurs de paramètres démographiques estimées, ne se trouvait pas dans la zone de paramètres concernée.

Dans le chapitre 4, nous avons montré que les populations de *V. canescens* sont susceptibles de subir deux types d'effet Allee génétique. L'un affecte le nombre de descendants fertiles par femelle et l'autre, le sex ratio. Nous avons utilisé une espèce dont la fécondité est élevée et qui exploite une ressource (les hôtes) dont la quantité fluctue et peut être très faible. Cela induit

probablement une forte compétition entre femelles, dans un contexte où il y a peu de source de mortalité autres que la compétition. Si le nombre de femelles diminue à cause de la production de mâles diploïdes, la compétition est réduite et chaque femelle peut produire plus de descendants. Il se peut donc la production de mâles diploïdes soit compensée par un plus grand nombre de descendants, et que l'effet Allee génétique affectant le nombre de descendants fertiles soit absent dans nos populations expérimentales. Dans ce cas, seul l'effet Allee affectant le sex ratio pourrait avoir un impact sur le taux d'accroissement des populations en rendant la population plus sensible à la stochasticité sur le sex ratio. Au vu des résultats, cet effet Allee génétique n'était pas assez fort comparé à l'impact de la stochasticité démographique pour qu'on observe un vortex d'extinction du à la production de mâles diploïdes.

Comme nous n'avons pas mis en évidence le « diploid male vortex » dans nos populations expérimentales, nous avons supposé que ces populations s'étaient éteintes à cause de la stochasticité démographique, présente dans toutes les petites populations. Mais l'intensité de la stochasticité démographique n'a pas été mesurée. Mesurer la stochasticité démographique dans une population régie par une dynamique proie-prédateur est compliqué par le fait que les variations des effectifs des populations de parasitoïdes sont aussi dues à la dynamique de la population d'hôte. Toutes les variables mesurées dans les populations pouvaient être expliquées par plusieurs modèles statistiques équivalents. Par exemple, pour l'analyse du temps avant extinction, le modèle avec l'AIC le plus faible incluait l'efficacité de recherche des parasitoïdes comme variable explicative. Pour l'analyse de l'efficacité de recherche, le modèle avec l'AIC le plus faible comportait le nombre de fondateurs. Mais dans les deux cas, la variable analysée pouvait aussi bien être expliquée par un modèle plus parcimonieux, que nous avons sélectionné. La production de mâles diploïdes pourrait donc avoir un impact sur les variables mesurée.

Il serait intéressant de refaire cette expérience en contrôlant plus de variables. On peut envisager d'utiliser un nombre fixe d'hôtes pour s'affranchir de la dynamique hôte-parasitoïde, ou de manipuler la force de la rétroaction hôte-parasitoïde, avec une partie des hôtes soumis à la dynamique hôte-parasitoïde, et une autre partie comportant un nombre fixe d'hôtes ajoutés régulièrement. Ceci permettrait d'observer l'impact de la dynamique hôte-parasitoïde sur la démographie des populations de parasitoïdes, comparées à des populations affranchies de cette dynamique. Les populations pourraient aussi être élevées en générations discrètes et non en générations chevauchantes, ce qui faciliterait l'analyse des taux d'accroissement et de la rétroaction entre populations d'hôtes et de parasitoïdes. Enfin, une manipulation du nombre d'hôtes parasités par femelle permettrait de contrôler l'intensité de la stochasticité démographique. Dans tous les cas, on s'éloignerait de la situation de populations naturelles afin de contrôler plus de variables et de mieux comprendre ce qu'il s'est passé dans l'expérience présentée ici.

6.3. Maintien de populations avec sl-CSD

6.3.1. Populations naturelles

Le sl-CSD peut être qualifié de « dessein inintelligent » (van Wilgenburg *et al.* 2006) car il peut entraîner la production de mâles diploïdes. Il apparaît donc comme un système moins optimal que d'autres systèmes de détermination du sexe présents chez les haplo-diploïdes, comme l'empreinte génomique (Dobson & Tanouye 1998). Pourtant, ce système, supposé ancestral chez les Hyménoptères, a été conservé chez certaines espèces, ce qui suggère que dans certaines circonstances, l'impact démographique de la production de mâles diploïdes n'est pas assez fort pour empêcher la survie de certaines populations. On ignore bien évidemment combien d'espèce à sl-CSD se sont éteintes à cause des conséquences démographiques de ce système.

La persistance de certaines espèces à sl-CSD peut être due à l'apparition de comportements adaptatifs limitant la production de mâles diploïdes ou son impact sur la dynamique des populations. Il s'agit par exemple de comportements de choix du partenaire : les femelles évitent l'accouplement avec des apparentés (Metzger *et al.* 2010), des mâles portant le même allèle qu'elles au gène du CSD (Thiel *et al.* 2013) ou des mâles diploïdes (Chapitre 4, on ne sait pas si la moindre probabilité d'accouplement des mâles diploïdes s'explique par un comportement de choix de la part des femelles si elle est intrinsèque aux mâles diploïdes). La dispersion avant accouplement limite les rencontres entre apparentés, surtout en cas de dispersion différée des mâles et des femelles (Mazzi *et al.* 2011). Au niveau individuel, la polyandrie limite la probabilité de produire des proportions très élevées de mâles diploïdes (Crozier & Page 1985). Enfin, la fertilité des mâles diploïdes (Cowan & Stahlhut 2004; Elias *et al.* 2009) permet aux femelles produisant des mâles diploïdes d'avoir la même fitness que les autres si elles se trouvent dans une grande population. Dans une petite population, la production de mâles diploïdes entraîne un biais de sex ratio et l'effet Allee génétique associé (Chapitre 4).

Malgré la présence de comportements de choix du partenaire chez *V. canescens* (Metzger *et al.*, 2010, Chapitre 4), les proportions de mâles diploïdes en populations naturelles sont non négligeables : elles vont de 3 à 15% (Chapitre 3) et sont similaires aux proportions observées dans des populations naturelles de *Cotesia glomerata* (10%, Ruf *et al.* 2013) et *C. rubecula* (15%, de Boer *et al.* 2012). Pour une population comportant 40% de mâles, comme rapporté dans des populations naturelles de *V. canescens* (Metzger *et al.*, 2008), ces proportions de mâles diploïdes impliquent que 4 à 24% des accouplements soient assortis, ce qui correspond à 2 à 12% des œufs diploïdes produits dans la population qui se développent en mâles stériles au lieu de femelles. Cette

réduction du nombre de femelles devrait avoir un impact sur le taux d'accroissement des populations. Il serait intéressant de relier les taux d'accroissement de populations naturelles à leur proportion de mâles diploïdes. Même si le taux d'accroissement reste positif (effet Allee démographique faible), il peut être réduit par la production de mâles diploïdes. Dans les populations où la compétition entre femelles est forte il se peut que la production de mâles diploïdes soit au moins partiellement compensée par une fitness accrue pour les femelles, qui souffrent moins de la compétition. Enfin, il est aussi tout à fait possible que certaines populations naturelles se soient éteintes à cause du « diploid male vortex», qui concerne de très petites populations (Zayed & Packer 2005). Si les populations naturelles comportant beaucoup de mâles diploïdes persistent avec un taux d'accroissement positif mais faible, elles peuvent être plus susceptibles de s'éteindre lors d'un changements des conditions environnementales (*e.g.* Belovsky *et al.* 1999).

6.3.2. Populations d'auxiliaires de lutte biologique

Les populations d'auxiliaires utilisées en lutte biologique classique sont fondées à partir d'un petit nombre d'individus prélevés dans une population naturelle, multipliés en élevages et relâchés dans une nouvelle zone géographique envahie par un ravageur, dans le but qu'ils contrôlent sa population. La production de mâles diploïdes peut affecter les populations lors des trois étapes principales : le prélèvement des individus fondateurs, la multiplication en laboratoire et le lâcher.

Lors du prélèvement d'individus fondateurs, si la population capturée comporte peu d'allèles au gène du CSD, des mâles diploïdes seront produits en laboratoire et dans la zone d'introduction. D'après une méta-analyse (Hopper & Roush 1993), le nombre de fondateurs capturés influence la probabilité d'établissement des populations de lutte biologique. Stouthamer *et al.* (1992) conseillent de prélever au moins 20 à 30 femelles sur le terrain pour que la population de laboratoire comporte tous les allèles d'une population naturelle qui en compte 20 donc les fréquences sont identiques, comme attendu pour ce locus (Yokoyama & Nei 1979). Ce nombre minimum de femelles à prélever est probablement plus élevé dans une population réelle, où les individus prélevés peuvent être apparentés et où les fréquences alléliques ne sont pas toujours à l'équilibre.

Lors de la phase d'élevage, des allèles au gène du CSD peuvent être perdus au cours du temps, ce qui augmente la production de mâles diploïdes et réduit le taux d'accroissement de la population (Stouthamer *et al.*, 1992). Comme le but de la phase d'élevage est d'augmenter la taille de la population de parasitoïdes, la production de mâles diploïdes réduit l'efficacité et augmente le coût de cette phase. Pour limiter la perte de diversité au gène du CSD, Stouthamer *et al.* (1992)

recommandent d'élever les auxiliaires de lutte biologique en une seule population de grande taille, en contrôlant si possible le nombre d'hôtes parasités par chaque femelle. Cook (1993) propose de créer une grande population et plusieurs petites populations qui servent de réservoir d'allèles régulièrement injectés dans la grande population. Introduire régulièrement dans la population d'élevage des individus capturés sur le terrain maintien aussi la diversité génétique de l'élevage (Cook 1993; Ode & Hardy 2008). La méthode proposée par Cook (sans introduction régulière d'individus du terrain) a été testée par Schneider & Vinuela (2007) sur le parasitoide *Hyposoter didymantor* dont le sexe est déterminé par sl-CSD. Elles ont obtenu une population comportant 40% de femelles, ce qui est jugé satisfaisant.

Après le lâcher des populations d'auxiliaires sur le terrain, la production de mâles diploïdes réduit le taux d'accroissement de la population et peut empêcher l'établissement de l'auxiliaire ou limiter l'efficacité du contrôle du ravageur. Le nombre d'individus par lâcher et le nombre total d'individus lâchés augmentent la probabilité d'établissement de l'auxiliaire (Hopper & Roush). Stouthamer *et al.* (1992) recommandent de constituer des populations de lâcher avec plusieurs populations d'élevage.

Les espèces avec sl-CSD sont peu représentées parmi les auxiliaires de lutte biologique. Les Braconidés et les Ichneumonidés, familles où le CSD est présent, ont une probabilité d'établissement plus faible que les Chalcidiens, chez qui le CSD n'a jamais été détecté (Hopper & Roush 1993; Stouthamer *et al.* 1992). Cependant, il semble que la production de mâles diploïdes ne soit pas la principale cause de cette observation, qui a été rapportée pour des nombres d'individus lâchés largement supérieurs au nombre recommandé pour éviter un fort impact de la production de mâles diploïdes sur l'établissement des populations. D'autres différences biologiques que le système de détermination du sexe, comme la durée d'une génération ou le stade de l'hôte attaqué, pourraient entrer en jeu (Hopper & Roush 1993). Certains programmes de lutte biologique impliquant des parasitoïdes à sl-CSD ont néanmoins fonctionné (*e.g. Habrobracon hebetor*, Prozell & Scholler 2003; Voloshenko & Khachumov 2000). De Boer *et al.*, (2012) ont mesuré une proportion de 15% de mâles diploïdes dans une population établie de l'auxiliaire *Cotesia rubecula*. Dans ce cas-là, la production de mâles diploïdes n'a pas empêché le succès de l'opération de lutte biologique, sans que l'on puisse savoir si cette situation est courante ou exceptionnelle.

6.4 Mise en évidence de processus génétique et démographiques

Les différents processus démographiques et génétiques menant à l'extinction des populations peuvent avoir lieux en même temps dans les populations. Il est donc parfois difficile de

dissocier leurs effets. L'étude de populations expérimentales permet en théorie de distinguer l'effet des deux types de processus : la taille et la diversité génétique des populations sont manipulées indépendamment l'une de l'autre. Cette approche est recommandée pour la détection d'effets Allee génétiques (Chapitre 2) et a été suivie pour l'expérience de test du « diploid male vortex » (Chapitre 5, pour d'autres exemples, voir Elam *et al.* 2007; Firestone & Jasieniuk 2013; Hufbauer *et al.* 2013). Elle comporte cependant des limites.

D'abord, la diversité génétique est le plus souvent manipulée à travers le nombre de familles fondatrices. Pour un même nombre de familles, on va donc créer des populations avec niveaux de taille de population. Dans les modalités où on utilise un plus grand nombre d'individus, on aura forcément une diversité génétique un peu plus élevée qui peut se traduire par un effet significatif de l'interaction entre les traitements « diversité génétique » et « taille de population ». On ne peut pas séparer complètement les deux types de processus car la stochasticité démographique entraîne fatalement de la stochasticité génétique (dérive) puisque les allèles sont portés par des individus, dont la nature entière génère la stochasticité démographique. Les organismes clonaux constituent une exception à cette situation et permettent une totale indépendance entre taille de population et diversité génétique (*e.g.* Firestone & Jasieniuk 2013).

Ensuite, pour certains designs expérimentaux, la diversité génétique initiale peut évoluer au cours du temps. Dans l'expérience de test du « diploid male vortex », on observe seulement une ingluence de la capacité de charge sur la probabilité d'extinction et on en déduit que ce n'est pas la production de mâles diploïdes qui entraîne l'extinction des populations car la diversité génétique initiale et les flux de gènes, qui sont censés influencer la diversité génétique au cours de l'expérience, n'ont pas d'effet sur l'extinction. Toutefois, peut-être que les flux de gènes étaient trop faibles pour influencer la diversité génétique, et que la diversité génétique initiale s'est réduite au cours du temps à cause des goulots d'étranglement causés par la dynamique hôte-parasitoïde et la capacité de charge. La capacité de charge serait alors le seul traitement ayant un impact sur la diversité génétique, d'où les résultats observés. Cette hypothèse me semble peu probable car la plupart des extinctions ont eu lieux peu après (0-16 semaines) la période utilisée pour la mesure des proportions de mâles diploïdes, qui était influencée par la diversité génétique initiale. De plus, les populations à capacité de charge faible comportaient moins de mâles diploïdes que les autres, il serait donc étonnant que cette tendance se soit inversée par la suite.

Identifier les causes génétiques et démographiques du déclin ou de l'échec d'établissement d'une population permet d'adapter les stratégies de gestion de cette population pour favoriser sa persistance. Si la population est affectée par un processus génétique, ses individus garderont une fitness faible même si les causes d'extinction extrinsèques à la population sont supprimées, ou si les

individus sont multipliés en captivité, alors que ces mesures seront souvent efficaces si la population est principalement affectée par la stochasticité démographique ou environnementale ou des effets Allee écologiques. Seule l'augmentation de la diversité génétique par migration pourra augmenter la fitness (rescousse évolutive, *e.g.* Madsen *et al.* 1996).

6.5 Conclusion

Au cours de cette thèse, nous avons décrits les effets Allee génétiques, un concept qui relie démographie et génétique. Nous avons mis en évidence un effet Allee génétique dû au système de détermination du sexe dans des populations expérimentales de *V. canescens*. Cet effet Allee génétique n'avait aucun impact sur la démographie des populations ou avait un impact plus faible que la stochasticité démographique. Ce travail souligne l'intérêt de l'étude de populations expérimentales, qui permet de distinguer les processus génétiques et démographiques menant à l'extinction des populations en manipulant indépendamment l'effectif et la diversité génétique de la population.

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ANNEXE : THE RESPONSE OF LIFE-HISTORY TRAITS TO A NEW SPECIES IN THE COMMUNITY: A STORY OF DROSOPHILA PARASITOIDS FROM THE RHONE AND SAONE VALLEYS

Cet article présente les résultats de mon stage de master réalisé en 2009 à l'université de Rennes 1. Même s'il n'est pas lié à mon sujet de thèse, j'y ai travaillé pendant ma thèse et des membres de mon laboratoire de thèse se sont impliqués dans l'analyse des résultats et la rédaction. C'est pourquoi je l'ai inclus en annexe.

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The response of life-history traits to a new species in the community: a story of *Drosophila* parasitoids from the Rhône and Saône valleys

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In the present study, we investigated the evolution of life-history traits in the main species of a community, after the arrival of a new competitor. Two parasitoid species, *Leptopilina heterotoma* and *Asobara tabida*, are present throughout the Rhône and Saône valleys, whereas a third species, *Leptopilina boulardi*, is slowly extending its distribution northwards. In the presence of *L. boulardi*, competing parasitoids experience a higher mortality and lower host availability. Resources should thus be re-allocated between traits according to these new factors. We compared life-history traits of populations of *L. heterotoma* and *A. tabida* in areas with and without *L. boulardi*. As predicted by both Price's balanced mortality hypothesis and the theory of life-history traits, we found that investment in reproduction is higher in southern populations for both native species, coupled with higher travelling abilities. However, only *A. tabida* paid their higher fecundity by a lower longevity. The absence of a clear trade-off between these traits in *L. heterotoma* may be explained by a lower metabolic rate in southern populations. These results highlight the importance of the community change over climate in the evolution of life-history traits in this parasitoid community. *Biological Journal of the Linnean Society*, 2012, **107**, 153–165.

ADDITIONAL KEYWORDS: *Asobara tabida* – competition – evolution – global change – *Leptopilina boulardi* – *Leptopilina heterotoma* – maintenance – mobility – reproduction – trade-off.

INTRODUCTION

Life-history refers to a set of strategies including behavioral, physiological, and anatomical traits (Ricklefs & Wikelski, 2002). These traits are crucial components of fitness and are therefore strongly influenced by natural selection. However, all possible combinations of life-history traits cannot occur in nature as a result of the existence of internal trade-offs: available resources are allocated preferentially to some traits to the detriment of others. One of the most classic trade-off arises between maintenance and reproduction (Ellers, van Alphen & Sevenster, 1998; Therrien *et al.*, 2008; Blomquist, 2009), although other traits such as dispersal can also be involved in trade-offs (Roff & Fairbairn, 1991; Zera & Denno, 1997; Hughes, Hill & Dytham, 2003; Gu, Hughes & Dorn, 2006). According to life-history

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theory, individuals should display an optimal allocation of resources between traits in a given environment (Roff, 1992; Stearns, 1992).

The influence of environment on life-histories results from the effect of both abiotic (e.g. climatic conditions) and biotic (e.g. community structure) variables (Stearns, 2000). Most of these abiotic and biotic variables that shape life-history traits are affected by the current change in climate occurring all over the globe (IPCC, 2007). The impact of climate change on life-histories is two-fold: there is a direct impact from abiotic changes and an indirect impact from biotic changes in reaction to new climatic conditions (Walther *et al*., 2002; Gienapp *et al*., 2008; Lepetz *et al*., 2009). Climate (or abiotic) change can thus bring biotic changes by modifying the resources (prey, food, hosts) distribution and availability, or by changing the distribution of competitors and natural enemies among others. These changes can induce modifications in the genotypes of organisms; this can be detected by comparing populations reared in the same environment for at least two generations (Kawecki & Ebert, 2004).

The *Drosophila* parasitoids from the Rhône and Saône valleys in France represent an interesting example of such indirect impact of climate change through a change in the community. North of the Rhône and Saône valleys, forming a 500-km long corridor, the community is mainly composed of the solitary larval endoparasitoids *Asobara tabida* Nees and *Leptopilina heterotoma* (Thompson). In the south, a third parasitoid, *Leptopilina boulardi* (Barbotin *et al*.), is present and dominant (Fleury, 1993; Fleury *et al*., 2004). The three species (constituting approximately 80% of the parasitoid community, Fleury *et al*., 2009) attack larvae of *Drosophila melanogaster* and *Drosophila simulans*, which represent two-thirds of the *Drosophila* in this area (Fleury *et al*., 2009). As a consequence of increasing temperature, *L. boulardi* is spreading northwards (Patot *et al*., 2010); its northern limit shifted of 60 km between 1997 and 2007 (R. Allemand, unpubl. data; Patot *et al*., 2010). *Leptopilina boulardi* is competitively superior to both *A. tabida* and *L. heterotoma*. When a host is parasitized by both *L. boulardi* and a native species, the former emerges more than 60% of the time against *L. heterotoma* (Fleury *et al*., 2000) and 70% of the time against *A. tabida* (Kraaijeveld, 1999). In addition, *Drosophila* hosts are exposed to higher levels of parasitism in the south (90% in Valence), where *L. boulardi* is present, than in the north (less than 60% in Lyon, 100 km north of Valence), where it is absent (Fleury *et al*., 2004); the proportion of healthy hosts available is thus lower in the south. The presence of *L. boulardi* results in a higher mortality rate and lower host availability for native species.

Based on Price's balanced mortality hypothesis (Price, 1974) stating that organisms suffering high levels of juvenile mortality should evolve a high fecundity, we expect, for native species, a higher fecundity in females of the populations in presence of the competitor. Because host availability is lower in presence of the competitor (Fleury *et al*., 2004), females of native species would gain by being able to fly further and being more active to find suitable hosts. However, because of a limited amount of resource that can be allocated to the different traits, we expect a lower longevity (Ellers, van Alphen & Sevenster, 1998).

Although the trade-off between fecundity and longevity has been observed in *A. tabida* (Ellers *et al*., 1998), it has not in *L. heterotoma* (Ris, 2003). It is thus important to measure other maintenance traits (e.g. lipid content and metabolic rate). Lipids are the main energy resource for parasitoids (Ellers, 1996; Bernstein & Jervis, 2008) and the metabolic rate is the rate of energy consumption by the organism. Similarly, not only fecundity, but also egg size should be measured. Differences in the amount of lipids, in metabolic rate or in egg size could result in no apparent trade-off between fecundity and longevity.

The present study aimed to investigate the impact of different length of exposition to a new competitor on the life-history traits of native species, and to detect possible subsequent trade-offs. To investigate this, two populations of each native species (*L. heterotoma* and *A. tabida*) were studied: one population was collected in an area where *L. boulardi* was first detected in 1997, and one population was collected in an area where it was first detected in 2006 (R. Allemand, pers. comm.). The sites were sufficiently close to reduce abiotic differences but sufficiently far apart for the competitor to arrive at different points in time. The main predictions for populations exposed for the longest time to the competitor are: (1) a higher fecundity because of a higher mortality; (2) a higher investment in mobility to increase host exploitation; and (3) a lower investment in maintenance as a result of the trade-off. To test these predictions, traits related to reproduction (egg load at emergence, egg size, lifetime potential fecundity, and ovigeny index), mobility (locomotor activity and wing loading) and maintenance (longevity, lipid content, and metabolic rate) were measured.

MATERIAL AND METHODS

Study sites

For both *A. tabida* and *L. heterotoma*, two populations were collected, 45 km apart, on a north–south transect in the Rhône and Saône valleys. The prox-

imity of the sites prevents any differences between them in host species available. Insects were collected in October 2008 using banana bait trapping. In all sampled populations, *L. boulardi* individuals also emerged from the traps. *A. tabida* was collected in Anse (45°56′33.42″N, 4°43′38.46″E; altitude 170 m) and Pacalon (45°32′57.90″N, 4°48′09.78″E; altitude 299 m); *L. heterotoma* in Saint-Bernard (45°56′42.90″N, 4°44′20.80″E; altitude 170 m) and Seyssuel (45°33′49.62″N, 4°49′18.78″E; altitude 216 m). The sites will be referred to as north (Anse and Saint-Bernard) and south (Pacalon and Seyssuel) for both species throughout the present study.

Abiotic factors

Using meteorological data from 2004 to 2009, mean monthly temperature and precipitation were compared for nearby sites: Reventin (45°28′42″N, 04°48′36″E; altitude 295 m), and Villefranche-sur-Saône (45°59′18″N, 04°43′24″E, altitude 212 m).

Rearing

To observe life-history differences as a result of genetic changes and not phenotypic plasticity, all populations were reared under the same laboratory conditions. Parasitoids were reared on a *Drosophila subobscura* population collected in the Netherlands in the late 1980s. The very low resistance of this host to parasitoid attacks (Eslin & Doury, 2006) eases parasitoid reproduction. Hosts and parasitoids were reared at 20 °C and 60% relative humidity. For host rearing, adults were allowed laying eggs in vials (height 13 cm, diameter 6 cm) containing a nutritive medium Agar-Kalmus-sugar-Nipagine surmounted by a small dome of living yeast. For parasitoid rearing, two 7–15-day-old couples were introduced in a vial (height 8 cm, diameter 5 cm) containing approximately 60 4-day-old host larvae on 1–1.5-cm Agar-Kalmus-Nipagine medium covered with living yeast. Hosts were reared until emergence of adult flies and wasps. Newly-emerged male and female parasitoids were kept in vials (10–30 parasitoids per vial) with agar medium and fed with 10% diluted honey.

General methods

All life-history traits were measured on females because they are the individuals facing the change in host availability. All experiments were conducted under a 12 : 8 h light/dark cycle at 20 °C and 60% relative humidity. For each individual used (except for measures of metabolic rate and wing loading for which the mass was taken), the length of the left hind tibia was measured with the numeric image analysis software PEGASE PRO, version 4 (2iSystem), under a binocular (×3.15; Olympus SZ-6045TR) linked to a video camera. Tibia length was shown to be a good proxy of individual size (Cronin & Strong, 1996; Nicol & Mackauer, 1999) and individual size is positively correlated with most life-history traits (Godfray, 1994). *Leptopilina heterotoma* is smaller than *A. tabida*.

Reproductive traits

Egg load and egg size at emergence

Egg load at emergence was measured by counting mature eggs in the ovarioles. Virgin females aged of less than 2 h were frozen at –80 °C and dissected under a binocular microscope (×4; Olympus BH2) ($N = 25$ for each population). A subsample of 30 eggs per females was photographed and the length (L) and width (w) measured using the numeric image analysis software IMAGEJ, version 1.14 (NIH) to estimate egg volume. For *A. tabida*, the volume (V) of eggs was calculated as the volume of two identical cones attached by their bases: $V = 1/12\pi Lw^2$. Eggs of *L. heterotoma* were considered as prolate spheres with a volume of $V = 1/6\pi Lw^2$. A mean egg volume was calculated for each female and used for the analyses.

Lifetime potential fecundity

An experiment was designed to estimate the number of eggs matured by a female throughout her life, hereafter defined as lifetime potential fecundity. All hosts used for this experiment were 4-day-old L2 larvae of *D. subobscura*. Mated females were kept in vials with food until they reached 7 days old. Because naive females may be reluctant to oviposition (van Lenteren, 1976; van Alphen & Drijver, 1982), each female was first tested for her motivation to oviposit: the female was placed into a Petri dish containing 24 host larvae. If no oviposition occurred after 15 min (less than one-third of females), the female was discarded from the experiment. If oviposition occurred, the female was used in the experiment. Between 15 and 17 females of each population of each species were placed individually into a rearing vial containing 150 host larvae for 8 h, and then transferred in a vial with food only for 24 h, allowing egg maturation (Ellers, Driessen & Sevenster, 2000). The same procedure was repeated: females were placed with 150 host larvae for 8 h, and were then transferred in vials with food until they were 15 days old. They were then frozen at –80 °C and dissected to count the number of mature eggs as previously described. All hosts in contact with females were reared until hosts or parasitoids emergence.

The lifetime potential fecundity was estimated as the sum of the total number of parasitoids emerging

from the hosts, the number of oocytes in the ovarioles at 15 days old and the estimated developmental mortality (C. Vayssade & N. Chazot, unpubl. data).

Ovigeny index

An ovigeny index was measured for each population of each species by calculating the proportion of the lifetime potential fecundity per population available at emergence (Jervis *et al*., 2001): for each population, the mean lifetime potential fecundity was divided by the mean egg load at emergence.

Mobility traits

Locomotor activity

The distance walked in 24 h was estimated for 14 7-day-old females per population. Females were placed individually into a Petri dish (diameter 4 cm) and videotaped. White lights were used during photophase (16 h) and red lights during scotophase (8 h). Females' position was recorded every 5 s using the software ETHOVISION, version 3.1 (Noldus information Technology), which calculates the distance walked, assuming that moves are straight between two positions successively recorded.

Wing loading

The wing loading is the ratio between the weight and the wing surface of an individual and gives an indication on the physical constraints it possesses: the lower the wing loading, the cheaper the flight (Angelo & Slansky, 1984; Starmer & Wolf, 1989; Gilchrist *et al*., 2004). For each population, 25 females aged < 2 h were frozen at –80 °C and weighed with a microbalance (Sartorius M4; ±0.001 mg). The area of the left wing was then measured using the numeric image analysis software PEGASE PRO, version 4, under a binocular (×3.15, Olympus SZ-6045TR) linked to a video camera.

Maintenance traits

Longevity

Longevity without food was measured on 50 females for both populations of *A. tabida*, and 66 and 59 females, respectively, for the northern and southern populations of *L. heterotoma*. To mimic what would normally happen under natural conditions, females were mated after emergence, and kept into a vial containing a substrate of Agar-Kalmus-Nipagine without food. Mortality was checked every day. Longevity without food was measured to document the amount of energy reserves available at emergence for maintenance, as well as the ability to use this energy.

Lipid content

The amount of lipids in the body of virgin unfed females aged < 2 h was measured using the colourimetric method *sensu* Giron *et al*. (2002). Twenty-four and 21 females, respectively, were used for the northern and southern populations of *A. tabida*, and 24 and 25 females for *L. heterotoma*.

Metabolic rate

The metabolic rate of parasitoids was measured by flow-through respirometry as the quantity of CO_2 released per hour at 20 °C. Seven-day-old females of each population ($N = 21$ per population for *A. tabida* and $N = 14$ per population for *L. heterotoma*) were tested during the photophase. Females were placed separately in small cylindrical chambers and CO_2 production was measured with an infrared CO_2 analyzer (CA-10A Carbon Dioxide Analyzer; Sable Systems International). Two tubes were left empty as controls. Four recordings of 5 min each were performed per individual and, because only one tube can be measured at a time, there was a lag of 80 min (5 min per tube) between two successive recordings. The first recording for each individual was discarded because individuals are usually stressed right after manipulation. The metabolic rate was calculated as the mean of the three last records. All individuals were weighted with a microbalance (Sartorius M4; ±0.001 mg).

Statistical analysis

Meteorological data from both localities (north and south) were compared using a two-way analysis of variance for repeated measures with mean monthly temperature and mean monthly precipitation as response variables and month and locality, and their interaction, as explanatory factors (GRAPHPAD PRISM, version 5.01; GraphPad Software Inc.). Differences in life-history traits between northern and southern populations of *A. tabida* and *L. heterotoma* were analyzed using generalized linear models. Locality, species, and tibia length, and all their interactions, were included in the model for the analysis of all traits except metabolic rate, for which mass was used instead of tibia length (Gillooly *et al*., 2001), and wing loading, for which only locality, species and their interactions were used. The distribution and link function for each variable were selected using goodness-of-fit criterion. A gamma distribution and inverse link function were used for distance walked, metabolic rate, wing loading, and volume of eggs. A gamma distribution and log link function were used for the amount of lipids. A negative binomial distribution and a log link function were used for the lifetime potential fecundity and egg load at emergence. A Weibull distribution and log link function were used for longevity. A model including all parameters and their interactions and all simpler models

Table 1. Most parsimonious generalized linear models for life-history traits of *Drosophila* larval parasitoids

	Variables and factors	χ^2	*P*
Reproduction	Egg load at emergence		
	Species	20.47	< 0.0001
	Population	22.70	< 0.0001
	Size	7.54	0.0060
	Species × Population	41.49	< 0.0001
	Potential fecundity		
	Species	13.72	0.0002
	Population	25.87	< 0.0001
	Size	51.46	< 0.0001
	Species × Size	36.96	< 0.0001
	Population × Size	6.77	0.0093
	Volume of eggs		
	Species	24.63	< 0.0001
	Population	10.45	0.0012
	Size	9.95	0.0016
Mobility	Locomotor activity		
	Population	6.06	0.0138
	Size	3.47	0.0625
	Wing loading		
	Species	104.13	< 0.0001
	Population	34.92	< 0.0001
	Species × Population	57.15	< 0.0001
Maintenance	Longevity		
	Species	2.86	0.0907
	Population	15.47	< 0.0001
	Size	44.97	< 0.0001
	Species × Population	23.41	< 0.0001
	Species × Size	6.21	0.0127
	Population × Size	12.96	0.0003
	Metabolic rate		
	Species	11.26	0.0008
	Population	3.21	0.0733
	Mass	4.30	0.0382
	Species × Population	5.24	0.0220
	Species × Mass	4.91	0.0267
	Amount of lipids		
	Species	24.16	< 0.0001
	Size	52.47	< 0.0001

Chi-squared and *P*-values are for the likelihood ratio of each parameter of the model for each variable tested.

Table 2. Most parsimonious generalized linear models for *Asobara tabida* and *Leptopilina heterotoma*

	Variables and factors	χ^2	*P*
Asobara tabida	Egg load at emergence		
	Population	65.90	< 0.0001
	Wing loading		
	Population	79.36	< 0.0001
	Longevity		
	Population	10.13	0.0015
	Size	11.78	0.0006
	Population × Size	7.57	0.0059
	Metabolic rate		
	No significant effect		
Leptopilina heterotoma	Egg load at emergence		
	Size	12.26	0.0005
	Wing loading		
	No significant effect		
	Longevity		
	Population	6.78	0.0092
	Size	82.58	< 0.0001
	Population × Size	6.56	0.0104
	Metabolic rate		
	Population	4.34	0.0373
	Mass	4.29	0.0383

These models concern only life-history traits for which the effect of population in global models was different in both species (significant effect of the interaction 'Species × Population'). Chi-squared and *P*-values are for the likelihood ratio of each parameter of the model for each variable tested.

were compared simultaneously using the Akaike information criterion (AIC). The model with the lowest AIC was selected. If several models were equivalent ($\Delta AIC < 2$), the most parsimonious model was selected. Within the selected model, we used the Wald chi-square statistic to test the significance of the different predictors ($\alpha = 0.05$). Tests were conducted with SAS software, version 9.1 (SAS Institute, Inc.). Because the present study aimed to compare life-history traits between the northern and the southern populations for two different species, only results concerning the effect of population as a main effect or in interaction with another factor are presented and discussed. Nonetheless, all other factors were included in the models for statistical rigour and are presented in Table 1. When the model selected contained a significant effect of the interaction 'population × species', the effect of population was analyzed on each species separately by building one general model per species (Table 2), in accordance with the protocol previously described.

RESULTS

Abiotic factors

Neither mean monthly temperature (month: $F = 182.8$, d.f. = 10, $P < 0.0001$; locality: $F = 0.754$, d.f. = 1, $P = 0.4107$; month × locality: $F = 0.008736$, d.f. = 10, $P = 1.000$), nor mean monthly precipitations (month: $F = 2.003$, d.f. = 10, $P = 0.0436$; locality: $F = 1.060$, d.f. = 1, $P = 0.3333$; month × locality: $F = 0.2686$, d.f. = 10, $P = 0.9863$) were significantly

Table 3. Comparison of life-history traits measured between northern (N) and southern (S) populations of *Asobara tabida* and *Leptopilina heterotoma*

	Traits	*Asobara tabida*	*Leptopilina heterotoma*
Reproduction	Egg load at emergence	**N < S**	N = S
	Potential fecundity	**N < S**	**N < S**
	Volume of eggs	**N < S**	**N < S**
	Ovigeny index	**N < S**	**N > S**
Mobility	Locomotor activity	**N < S**	**N < S**
	Wing loading	**N > S**	N = S
Maintenance	Longevity	**N > S**	?
	Metabolic rate	N = S	**N > S**
	Amount of lipids	N = S	N = S

Significant differences between populations within a species are shown in bold.

different between the north and south. Thus, no major differences were present between the northern and the southern populations. Therefore, abiotic factors should have no direct effect on the difference in life-history traits between populations.

Life-history traits

All statistics from global models are presented in Table 1 and statistics from models for each species are presented in Table 2. Results of interest are summarized in Table 3. All values in the text are given as the mean ± SD.

Reproductive traits

The number of mature eggs present in the ovarioles at emergence was higher in the southern population for *A. tabida* (161.68 ± 21.25 eggs versus 76.52 ± 24.13 eggs) but no difference was observed for *L. heterotoma* (146.16 ± 65.63 eggs versus 166.96 ± 55.15 eggs) (Fig. 1A). However, for both species, females from the south had bigger eggs at emergence ($1.14 \pm 0.25 \times 10^{-4}$ mm^{-3} for *A. tabida* and $1.48 \pm 0.35 \times 10^{-4}$ mm^{-3} for *L. heterotoma*) than females from the north ($1.04 \pm 0.27 \times 10^{-4}$ mm^{-3} for *A. tabida* and $1.25 \pm 0.29 \times 10^{-4}$ mm^{-3} for *L. heterotoma*) (Fig. 1B). In both species, the lifetime potential fecundity depended on the females' size. However, for both species, and especially for *A. tabida*, in most of the size range of both populations, the model predicted a higher lifetime potential fecundity in the southern population compared to the northern one (Fig. 2). As predicted, reproductive investment was then higher in the south for both species. Finally, the ovigeny index is higher in the south in *A. tabida* (0.36 versus 0.21) but lower in the south in *L. heterotoma* (0.31 versus 0.43).

Mobility

The distance walked by females was higher for the southern population than for the northern one in both species (Fig. 3A). *Asobara tabida* females from the south had a lower wing loading (0.39 ± 0.07 mg mm^{-2}) and so a better flying ability at emergence than females from the north (0.67 ± 0.08 mg mm^{-2}). However, no significant effect of population on wing loading was detected for *L. heterotoma* (Fig. 3B). As predicted, for both species, females from the south thus invested more in mobility and this difference was more important in *A. tabida* than in *L. heterotoma*.

Maintenance

For both species, longevity was significantly influenced by the population but there was also an interaction with size. The effect of the population on longevity is then different, depending on the females' size. However, for *A. tabida*, in the size range of both populations, the model predicted a shorter longevity in the southern population compared to the northern one (Fig. 4A). For *L. heterotoma*, no significant difference was observed between both populations (Fig. 4A).

For both species, there was no difference in the amount of lipids at emergence between northern and southern populations: *A. tabida* females had 105.59 ± 40.95 μg of lipids per mm of tibia in the southern population and 186.80 ± 80.89 μg mm^{-1} in the northern one. These values were of 124.35 ± 66.95 μg mm^{-1} in the south and 137.43 ± 82.78 μg mm^{-1} in the north for *L. heterotoma*. The metabolic rate of females was lower in the population from the south (54.81 ± 16.76 μL of CO_2 rejected per minute) than in the population from the north (66.99 ± 27.12 μL min^{-1}) in *L. heterotoma*, but not in *A. tabida* (103.58 ± 19.94 μL min^{-1} in the south and 99.70 ± 27.84 μL min^{-1} in the north) (Fig. 4B).

DISCUSSION

As predicted by Price's balanced mortality hypothesis, lifetime potential fecundity, egg load at emergence, and egg size were higher in the southern population for *A. tabida*. For *L. heterotoma*, the southern population (in most of its size range) had bigger eggs and a higher lifetime potential fecundity, although no difference was found in egg load at emergence. Thus, investment in reproduction was higher in the south than in the north for both species and this difference affected more traits for *A. tabida* than for *L. heterotoma*. Although an increased investment in fecundity in the south has already been described for both *A. tabida* (Ellers & van Alphen, 1997) and *L. heterotoma* (Ris *et al.*, 2004), these studies were made on

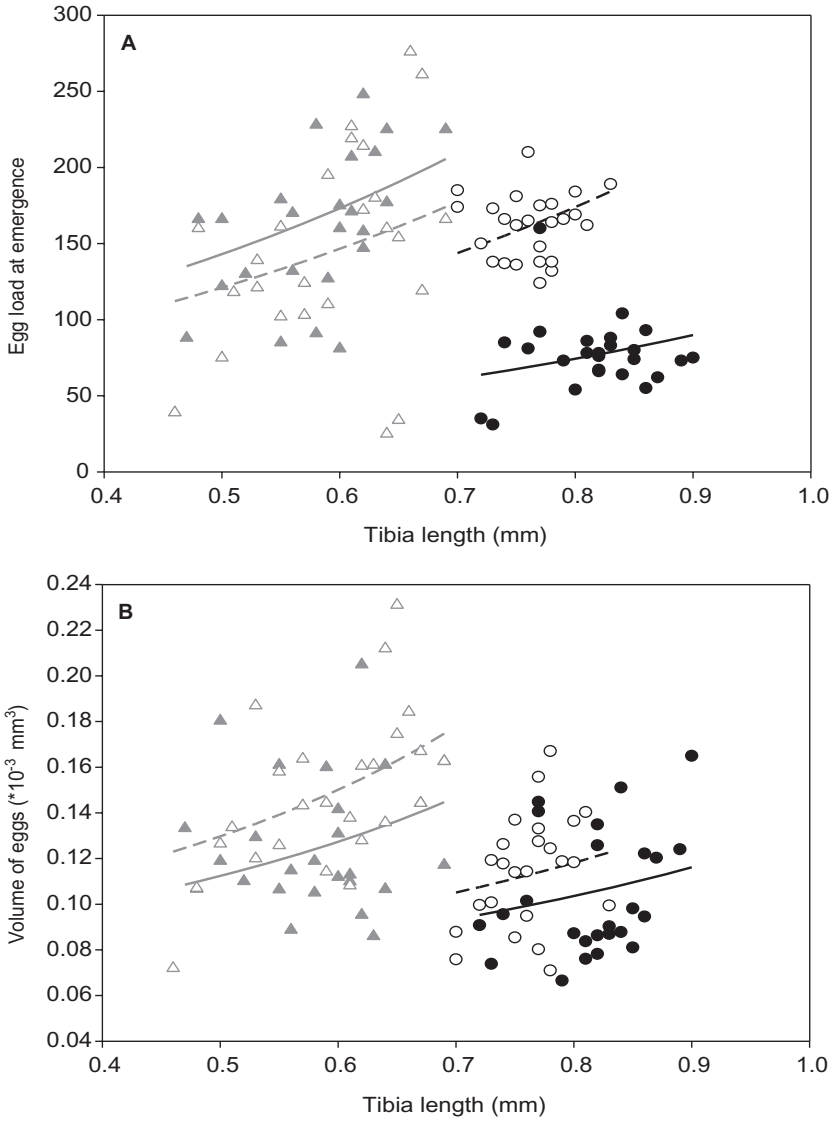

Figure 1. Reproductive traits at emergence. Egg load at emergence (A) and volume of eggs (B) as a function of female size. Circles represent observed values for *Asobara tabida* and triangles represent *Leptopilina heterotoma*. Open symbols represent the southern population and filled symbols represent the northern population. Dotted lines represent the predictions of the generalized linear models for southern populations and continuous lines represent northern populations.

larger scales, in contrast to the present study. In these studies, the environmental factors responsible for this difference were hypothesized as habitat distribution, climate or host species. In the present study, these factors have been excluded by the proximity of the study sites; no significant difference in temperature and precipitation was shown between the north and south of the study area. The proximity of the study sites also prevents any major difference in the host species distribution. In addition, *L. heterotoma* populations are highly genetically differentiated, suggesting low gene flow and very local adaptations (Fleury *et al.*, 2004). The main difference between populations is thus the date of arrival of *L. boulardi*, although we cannot exclude the possibility that other unmeasured factors also play a role (e.g. host switching, niche separation, geographical barriers, etc.).

The trade-off generally observed between egg size and number (Godfray, 1994) has not been observed in the present study: both species have a higher lifetime potential fecundity and bigger eggs in the south. Females probably benefit from producing larger eggs; larger eggs increase larval weight (Boivin & Gauvin,

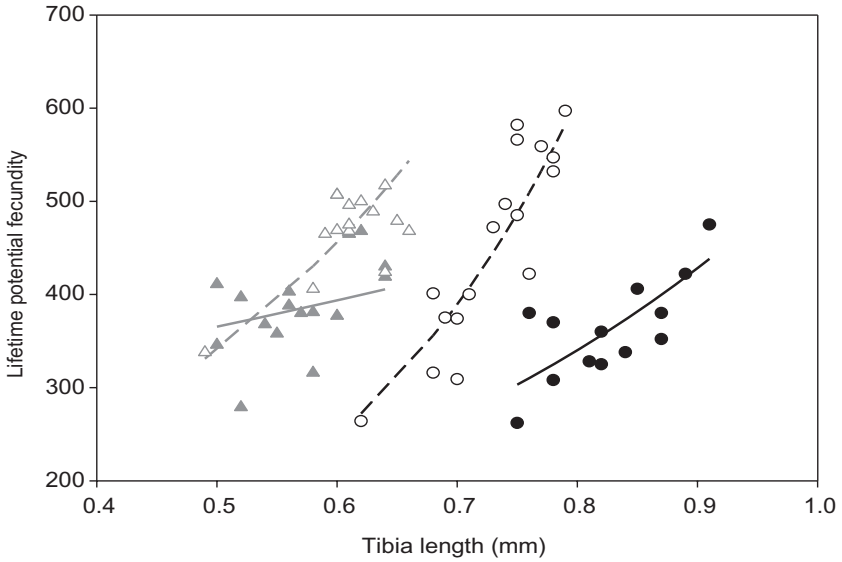

Figure 2. Lifetime potential fecundity as a function of female size. Circles represent observed values for *Asobara tabida* and triangles represent *Leptopilina heterotoma*. Open symbols represent the southern population and filled symbols represent the northern population. Dotted lines represent the predictions of the generalized linear models for southern populations and continuous lines represent northern populations.

2009) and higher larval weight is more advantageous under competition (Parker & Begon, 1986).

Both species also potentially have better mobility abilities in the southern populations, as expected. Because southern populations are assumed to be more adapted to the lower host availability resulting from the higher parasitism rate of *Drosophila* larvae (Patot *et al*., 2010), females would benefit from being able to travel to search for available hosts.

Locomotor activity is a measure of the general activity of the insect. An insect that is highly active and walks long distances is considered to have a high propensity to disperse (Pompanon, Fouillet & Boulétreau, 1999; Fleury *et al*., 2009). Females from both species were more active in the south than in the north. Such a higher activity rate in the south has also been observed in *L. heterotoma* on a larger scale (Fleury *et al*., 2009) and was proposed to be an adaptation to competition with *L. boulardi* linked to differences between species in daily rhythm of activity. Indeed, *L. heterotoma* is active earlier in the morning than *L. boulardi*. Because a higher activity rate enables to lay eggs quicker, more active females lay more eggs in the morning and larvae gain a competitive advantage by being several hours older than *L. boulardi* larvae laid subsequently in the same host (Fleury *et al*., 1995).

Wing loading was lower (i.e. flight is less expensive) for southern *A. tabida* females but not for *L. heterotoma*. This can show a difference between short- and long-distance travelling by individuals: walking is a cheaper method of covering short-distance than flying (Nation, 2008) (e.g. between two fruits or two branches of the same tree) but flying is more appropriate for long-distance (e.g. between trees or between orchards). In the laboratory, *L. heterotoma* has a much lower propensity to fly, and appears to be travelling more often by walking, in contrast to *A. tabida*, which tends to fly more often (V. Martel, pers. observ.). A lower propensity to fly could then explain that no difference in wing loading was found between both populations of *L. heterotoma*: the higher locomotion activity in the south is sufficient to enable females to travel more. In the present study, a higher mobility can allow for more widely spaced dispersion of eggs in a habitat where some patches might subsequently be parasitized by a superior competitor, or where healthy hosts could be scarcer because of the presence of an additional parasitoid species. In addition, such higher mobility can allow exploitation of more hosts in populations where the investment in reproduction is higher. However, further studies would be needed to measure the dispersal abilities of these populations.

Having an increased investment in reproduction and mobility incurs some costs because the energy invested in these traits cannot be invested in other traits (Ellers *et al*., 2000; Zhao & Zera, 2006). In *A. tabida*, females from the southern population had a higher lifetime potential fecundity, larger eggs, and better mobility abilities than females from the northern populations, which resulted in a shorter

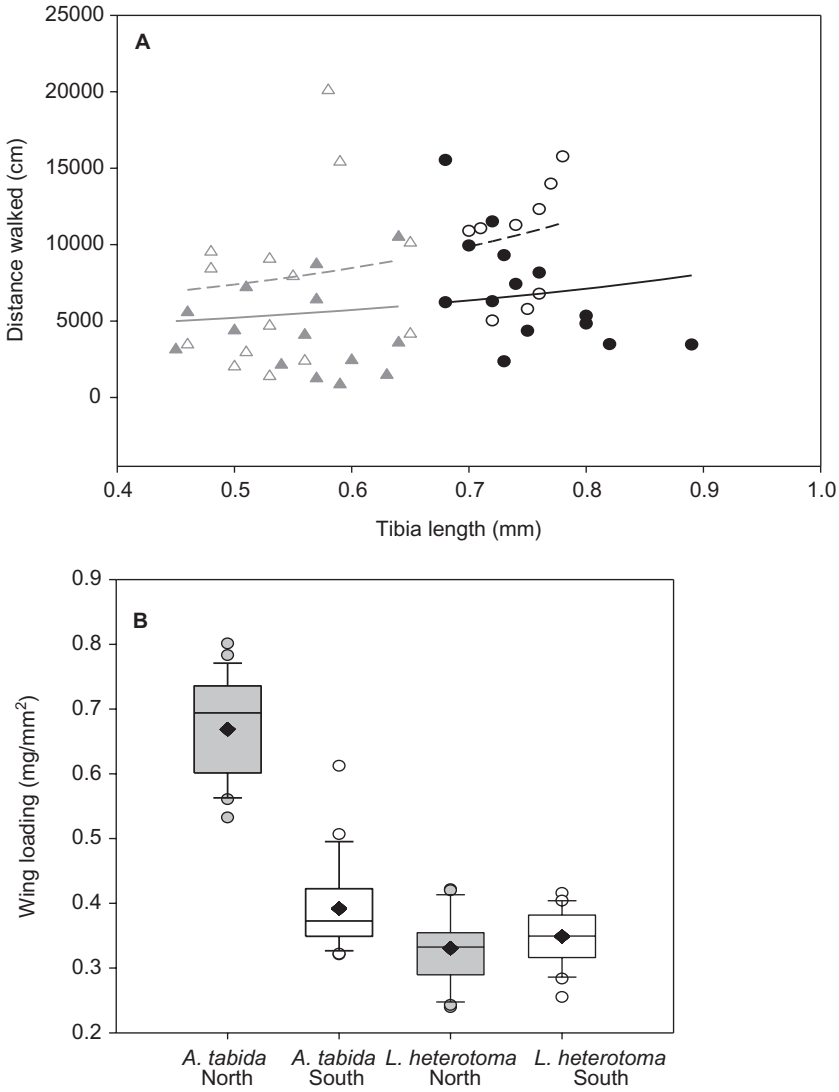

Figure 3. Mobility traits. A, locomotor activity as a function of female size. Circles represent observed values for *Asobara tabida* and triangles represent *Leptopilina heterotoma*. Open symbols represent the southern population and filled symbols represent the northern population. Dotted lines represent the predictions of the generalized linear models for southern populations and continuous lines represent northern populations. B, wing loading. Box plots represent observed values and black diamonds represent the predictions of the generalized linear model.

longevity, not only demonstrating the classical trade-off between reproduction and longevity (Ellers *et al*., 2000; Creighton, Heflin & Belk, 2009), but also a trade-off between mobility and longevity. In addition, Jervis *et al*. (2001) predicted and showed a negative correlation between ovigeny index and life span, as observed in *A. tabida* in the present study, as well as in a previous study (Ellers & van Alphen, 1997). In *L. heterotoma*, females from the southern population also had a higher lifetime potential fecundity (in most of their size range), produced larger eggs, and had better mobility abilities. Such an increase in the investment in reproduction and mobility should normally be countered by a shorter lifespan than northern females. However, these females also had a lower ovigeny index, thus predicting, in opposition to the theory of trade-offs, a higher longevity (Jervis *et al*., 2001). In the present study, we did not detect any clear difference in longevity between *L. heterotoma* populations, as in a previous study (Ris, 2003). However, the present study showed a significant difference in the metabolic rate between the two *L. heterotoma* populations tested: females from the south had a lower metabolic rate than females from the north. The metabolic rate is a measure of the speed at which energy is used (Brown *et al*., 2004) and,

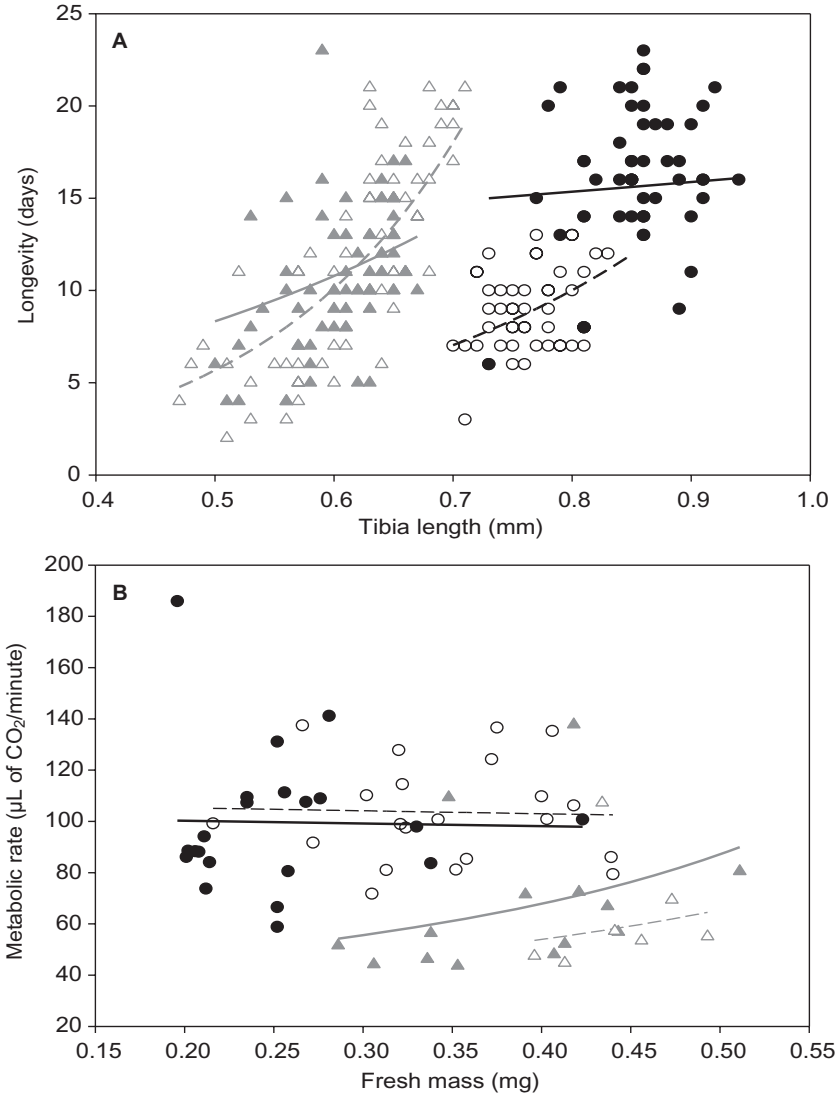

Figure 4. Longevity (A) and metabolic rate (B) as a function of female size. Circles represent observed values for *Asobara tabida* and triangles represent *Leptopilina heterotoma*. Open symbols represent the southern population and filled symbols represent northern population. Dotted lines represent the predictions of the generalized linear models for southern populations and continuous lines represent northern populations.

although longevity was shown to be negatively correlated to metabolic rate across five *Asobara* species (Seyahooei, van Alphen & Kraaijeveld, 2011), no such correlation was observed in *L. heterotoma*. The opposite predictions of the trade-off between fecundity and longevity and of the ovigeny index, coupled with a lower metabolic rate in southern populations of *L. heterotoma*, could thus result in a higher investment in reproduction and mobility without reducing longevity. By contrast, this difference in metabolic rate was lacking in *A. tabida* where the data were in agreement with a trade-off between reproductive investment and longevity, as well as with the predictions related to the ovigeny index.

Although both *A. tabida* and *L. heterotoma* showed changes in life-history traits congruent with predictions, these changes were more important for *A. tabida* than for *L. heterotoma*: in contrast to *A. tabida*, northern and southern populations of *L. heterotoma* did not display differences in egg load at emergence and wing loading. Differences in temporal niches and host ranges between *L. heterotoma* and *L. boulardi* are a possible explanation for this pattern, with such differences being less important for *A. tabida*. Indeed, during the winter, *L. heterotoma* has quiescence as adult (Eijs, 1999), whereas *A. tabida* and *L. boulardi* enter diapause as pupa (Baker, 1979) and late larva (Claret & Carton, 1980),

respectively. This results in different dates of emergence in spring, with *L. heterotoma* emerging first, followed by *A. tabida* and then *L. boulardi*. *Leptopilina heterotoma* thus remains longer than *A. tabida* without suffering from competition by *L. boulardi*. Second, *L. heterotoma* is more generalist than *A. tabida* and *L. boulardi*, attacking more than ten species of *Drosophila* and related genera, whereas *L. boulardi* is a specialist of *D. melanogaster* and *D. simulans* and *A. tabida* develops on *D. subobscura*, *D. obscura*, and *D. melanogaster* (Fleury *et al*., 2009). Thus, in the areas where the number of *D. melanogaster* and *D. simulans* hosts available is reduced by the presence of *L. boulardi*, *L. heterotoma* can still use alternative host species if available.

Although numerous traits were measured for each population, only one population per species and locality were analyzed. We thus cannot exclude the possibility that particular local conditions (e.g. habitat structure, composition of host community) are, at least partially, responsible for the observed values. However, some traits display the same difference for both species, which strengthen the hypothesis that they are the consequence of the presence of *L. boulardi*.

In conclusion, changes in the composition of communities appear to have selected for evolution in life-history traits of two native parasitoid species sharing common resources. Because the temporal and ecological niche of *L. boulardi* overlaps more with the one of *A. tabida* than with the one of *L. heterotoma*, this last species displays fewer changes in life-history traits in presence of the competitor.

These shifts (i.e. increased reproduction and better mobility) would likely be adaptive in an environment where juvenile mortality is higher by increasing the probability of finding hosts and of having progeny successfully developing. However, because both *L. heterotoma* and *A. tabida* are still present in the south of the Rhône Valley even 10 years after the arrival of *L. boulardi* (R. Allemand, pers. comm.), these modifications consequently most likely bring sufficient benefits for the populations to maintain themselves. Besides, in the south of the Rhône Valley, a high proportion of *L. boulardi* females (from 55% to 95% according to populations) are infected with the LbFV virus, which decreases their competitiveness by increasing their propensity to lay eggs in already parasitized hosts (Patot *et al*., 2010). Laboratory experiments have shown that the very high prevalence of LbFV virus in the competitor, *L. boulardi*, enables the coexistence of both *Leptopilina* species (Patot *et al*., 2012).

Another suitable reaction of native species to competition would be to extend their habitat by exploiting habitats not preferred by *L. boulardi*, thus decreasing the competition. Sampling habitats non-exploited by *L. boulardi*, or hosts not parasitized by it, could provide information on a possible habitat shift for the species being less successful.

Studying a local population of both native species at different points in time could help determine whether the change in life-history traits observed is really caused by adaptation to the new competitor. This would also remove the possible effect of the microhabitat by sampling at the same location every year. Finally, similar studies should be conducted on different communities of parasitoid species, initially allowing these findings to be generalized to all parasitoid communities, and eventually at a broader scale.

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